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Efeitos Comportamentais e GABAérgicos da Autoadministração Oral de Cocaína em Ratos Machos e Fêmeas com Transtorno de Déficit de Atenção e Hiperatividade Induzido por 6-Hidroxidopamina

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RESUMO

O Transtorno de déficit de atenção e hiperatividade (TDAH) é caracterizado por hiperatividade, desatenção e impulsividade, resultando de uma possível anormalidade cerebral com prejuízos nos sistemas noradrenérgico e dopaminérgico. Há diferenças entre os sexos nos sintomas de TDAH. Os meninos tendem a ser mais hiperativos e impulsivos enquanto as meninas são mais desatentas. Uma das comorbidades mais prevalentes é o transtorno por uso de substâncias. Indivíduos com TDAH têm risco de dependência a drogas, principalmente cocaína e cerca de um quarto dos dependentes possuem TDAH. Uma das explicações para a associação são os efeitos paradoxais de psicoestimulantes, que reduzem os sintomas da doença. O modelo de lesão dopaminérgica neonatal por 6-hidroxidopamina (6-OHDA) é utilizado para o estudo do TDAH, causando aumento da atividade locomotora em machos. O efeito paradoxal caracteriza-se por uma diminuição na locomoção após autoadministração de cocaína. Em humanos e em animais normais as drogas de abuso afetam os sexos de forma diferente, com os indivíduos do sexo feminino progredindo mais rapidamente para a dependência com maior resposta aos efeitos da administração repetida de cocaína. Estudos sugerem que as mulheres com TDAH apresentariam um maior risco de abuso de cocaína. A disfunção dopaminérgica e noradrenérgica pode interferir nos sistemas glutamatérgico e GABAérgico. O modelo de lesão dopaminérgica causa alterações na expressão de RNAm de subunidades $GABA_A$ no córtex pré-frontal, hipocampo e estriado. A exposição subaguda à cocaína modifica a liberação de GABA na área tegmental ventral e altera os níveis de GABA extracelular no córtex pré-frontal. Há escassez de estudos neurobiológicos sobre as diferenças sexuais em relação ao TDAH e ao uso de cocaína e as possíveis implicações do sistema GABAérgico, bem como sobre a comparação de autoadministração de cocaína em machos e fêmeas em modelos animais de TDAH. Assim, o objetivo deste estudo foi verificar os efeitos da autoadministração oral de cocaína no comportamento de ratos machos e fêmeas com TDAH induzido por lesão com 6-OHDA e a associação correspondente com a expressão de RNAm das subunidades dos receptores $GABA_A$ no córtex pré-frontal. Este estudo demonstra que o TDAH diminui os reforços na autoadministração de cocaína. Também foi possível concluir que os machos com TDAH possuem níveis de reforço menores que as fêmeas ao longo da autoadministração. Não houve

alterações significativas nas subunidades α1, α2 e γ2 do $GABA_A$. Porém, embora não significativo, os machos com TDAH tendem a apresentar um padrão diferente dos demais grupos de acordo com a subunidade. Existe correlação entre a expressão de RNAm das diferentes subunidades nas fêmeas normais e correlação do comportamento de reforço com a subunidade α2 nos machos com TDAH. Nossos resultados fornecem informações importantes quanto aos padrões de reforço de drogas em animais induzidos ao transtorno e a implicação do sexo nesses padrões. Demonstra que o efeito paradoxal da cocaína não acontece quando há um alto consumo desta pelos animais com TDAH. Quanto à expressão das subunidades do receptor $GABA_A$, nossos resultados indicam que pode haver modificações importantes ocasionadas pelo consumo de cocaína em animais com TDAH. Portanto percebe-se a necessidade de mais estudos que verifiquem o consumo de cocaína em animais machos e fêmeas induzidos ao TDAH e as alterações da expressão de subunidades do $GABA_A$ relacionados com adição a psicoestimulantes para elucidar as bases comportamentais e neuroquímicas da coexistência desses dois transtornos.

Palavras-chave: TDAH, autoadministração de cocaína, diferenças sexuais, $GABA_A$, córtex pré-frontal.

ABSTRACT

Attention deficit hyperactivity disorder (ADHD) is characterized by hyperactivity, inattention and impulsivity, resulting from a possible brain abnormality with dysfunction in noradrenergic and dopaminergic systems. There are gender differences in ADHD symptoms. Boys tend to be more hyperactive and impulsive while girls are more inattentive. One of the most prevalent comorbid disorder is substance use. Individuals with ADHD have higher risk for drug dependence, mainly cocaine, and about a quarter of the dependents have ADHD. One explanation for the association is the paradoxical effects of psychostimulants, which reduce the symptoms of the disorder. The neonatal dopaminergic lesion model by 6-hydroxydopamine (6-OHDA) is used for the study of ADHD, resulting in increased locomotor activity in males. The paradoxical effect characterized by a decrease in locomotion after cocaine self-administration. In normal humans and animals drugs of abuse affect sexes differently, with females for progressing faster dependence and greater response to the effects of repeated administration of cocaine. Studies suggest that women with ADHD present a greater risk of cocaine abuse. The dopaminergic and noradrenergic dysfunction can interfere with glutamatergic and GABAergic systems. The model of dopaminergic lesion causes changes in $GABA_A$ subunits of mRNA expression in prefrontal cortex, hippocampus and striatum. Subacute exposure to cocaine modifies the release of GABA in the ventral tegmental area and changes the extracellular GABA levels in the prefrontal cortex. There is an insufficient of neurobiological studies of sex differences in relation to ADHD and the use of cocaine and the possible implications of the GABAergic system and on the comparison of cocaine self-administration in males and females in ADHD animal models. The objective of this study was to investigate the effects of oral cocaine self-administration in male and female rats with ADHD-induced lesion by 6-OHDA and the corresponding association with the mRNA expression of the subunits of the $GABA_A$ receptors in the prefrontal cortex. This study demonstrates that ADHD causes decreases in self-administration of cocaine. It was also concluded that males with ADHD have lower reinforcement than females in oral cocaine self- administration. There were no significant changes in subunits $\alpha 1$, $\alpha 2$ and $\gamma 2$ $GABA_A$. However, although not significant, males with ADHD tend to have a different pattern from the other groups in accordance with the subunit. There is a correlation between the

mRNA expressions of the different subunits in normal females and there is a correlation between reinforcement and subunit $\alpha 2$ in males with ADHD. Our results provide important information about the cocaine reinforcement patterns in animals induced the disorder and the implication of sex in these standards. It shows that the paradoxical effect of cocaine does not happen when there is a high consumption of the animals with ADHD. The expression of the $GABA_A$ receptor subunits, our findings indicate that there may be significant changes caused by cocaine in animals with ADHD. So we see the need for more studies to verify the consumption of cocaine in male animals and induced ADHD females and changes in $GABA_A$ subunit expression related addition to psychostimulants to elucidate the behavioral and neurochemical bases of the coexistence of these two disorders.

Keywords: ADHD, cocaine self-administration, sex differences, $GABA_A$, Prefrontal cortex

LISTA DE ABREVIAÇÕES

TDAH – Transtorno de Déficit de Atenção e Hiperatividade

DSM V – Diagnostic and Statical Manual of Mental Disorders V

6-OHDA – 6 Hidroxidopamina

CPF- Córtex pré-frontal

ADAD - Abuso e Dependência de Álcool e outras Drogas

SNC – Sistema Nervoso Central

UFCSPA – Universidade Federal de Ciências da Saúde de Porto Alegre

EUA – Estados Unidos da América

HCl – Ácido Clorídrico

HBr – Ácido Bromídrico

HCO3 – Bicarbonato

H2O2- Peróxido de Hidrogênio

DA- Dopamina

DAT- *Dopamine Transporter*

GABA – Ácido gama - aminobutírico

SHR - *Spontaneously Hypertensive Rat*

WKHA - *Wistar-Kyoto hyperactive*

Brdu- 5-bromo-2'deoxiuridina

MAO- Monoamina-oxidase

ROS- Espécies Reativas de Oxigênio

NAc- Núcleo *accumbens*

ONU – Organização das Nações Unidas

NSDUH – *National Survey in Drug Use and Health*

UNODC - *United Nations Office on Drugs and Crime*

PND- *Postnatal day*

mRNA- *messenger Ribonucleic Acid*

ADHD – *Attention Deficit Hyperactivity Disorder*

VTA – *Ventral Tegmental Area*

SUD – *Substance Use Disorder*

FR1- *Fixed ratio 1*

mPFC – *medial PreFrontal Cortex*

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1. INTRODUÇÃO

1.1 TRANSTORNO DE DÉFICIT DE ATENÇÃO E HIPERATIVIDADE

1.1.1 Definição e Epidemiologia

O Transtorno de Déficit de Atenção e Hiperatividade (TDAH) consiste em uma desordem associada a comportamento de desatenção e/ou hiperatividade e impulsividade e afeta principalmente crianças em idade escolar, interferindo no funcionamento e no desenvolvimento (AMERICAN PSYCHIATRIC ASSOCIATION, 2014; SADOCK & SADOCK, 2007). Esse transtorno é consequência de uma possível anormalidade cerebral com disfunções no sistema catecolaminérgico, envolvendo especialmente a dopamina e noradrenalina (PLISZKA, 2005; SADOCK & SADOCK, 2007; ROHDE & HALPERN, 2004). A disfunção nesse sistema resulta na incapacidade de manter a atenção por um período de tempo adequado, comportamento impulsivo aumentado, capacidade diminuída de suprimir atividade motora e tendência à distração (PLISZKA, 2005; LAMBERT & KINSLEY, 2006; POETA & NETO, 2004) que leva a dificuldades cognitivas e de aprendizado. De acordo com o DSM-V, o TDAH é subdividido em três tipos: a) TDAH com apresentação predominantemente desatenta; b) TDAH com apresentação predominantemente hiperativa/impulsiva; c) TDAH com apresentação combinada, quando tanto os critérios de desatenção quanto de hiperatividade-impulsividade são preenchidos (AMERICAN PSYCHIATRIC ASSOCIATION; 2014; NUSSBAUM, 2011). A desatenção manifesta-se comportamentalmente como divagação em tarefas, falta de persistência, dificuldade em manter o foco e desorganização. A hiperatividade em crianças refere-se à atividade motora excessiva quando não apropriado, enquanto nos adultos há manifestação de inquietude extrema, levando a dificuldades de relacionamentos, pois há esgotamento dos amigos e familiares com este excesso de atividade motora. A impulsividade refere-se a ações precipitadas, que ocorrem no momento, sem premeditação ou planejamento e com elevado potencial para dano à pessoa, também podendo ser reflexo de um desejo de recompensas imediatas ou de incapacidade de postergar a gratificação (AMERICAN PSYCHIATRIC ASSOCIATION; 2014). Os sintomas do TDAH, geralmente, se expressam antes dos 7 anos, não podendo-se excluir a possibilidade do aparecimento dos sintomas após

essa idade (ROHDE & HALPERN, 2004). Ao longo da vida, a hiperatividade, normalmente, regride, porém na adolescência ou na fase adulta a maioria dos pacientes permanece com as dificuldades de planejamento, inquietude, desatenção e impulsividade (AMERICAN PSYCHIATRIC ASSOCIATION, 2014). Quando há resquícios de TDAH em adultos, estes apresentam deficiências em uma série de domínios cognitivos em relação à atenção sustentada, funções executivas, memória de trabalho e inibição de respostas. Estas características podem implicar em tomada de decisões desfavoráveis e, consequentemente, num risco aumentado para o abuso de substâncias, direção perigosa, instabilidade profissional e dificuldades no sustento de relação estáveis (AGAY, et al, 2010).

A prevalência do TDAH na população está estimada em 5% nas crianças e 2,5% nos adultos (AMERICAN PSYCHIATRIC ASSOCIATION, 2014). Há maior predominância em meninos do que em meninas, sendo a proporção de 2 para 1 em crianças e de 1,6 para 1 nos adultos conforme a recentre publicação do DSM-V (AMERICAN PSYCHIATRIC ASSOCIATION, 2014). Essa proporção parece ter mudado, uma vez que se acreditava que, no geral, esta desordem fosse de 3 a 4 vezes mais comum no sexo masculino (BIEDERMAN & FARAONE, 2005; GAUB E CARLSON, 1997). A estas mudanças pode-se atribuir um provável aumento do diagnóstico no sexo feminino.

Em desacordo com as estatísticas acima, um estudo que avaliou alunos com idades entre 6 e 12 anos de escolas na Venezuela, mostrou uma incidência maior de TDAH no sexo feminino (8,26%) em comparação com o sexo masculino (6,20%), evidenciando um desacordo com a literatura especializada (MONTIEL-NAVA *et al*, 2002).

A diferença entre os sexos pode ser resultado da dificuldade do diagnóstico do transtorno no sexo feminino, devido, provavelmente, ao fato de as meninas apresentarem menos sintomas de conduta, como hiperatividade e impulsividade, causando menos incômodo às famílias e à escola e, portanto, fazendo com que sejam menos encaminhadas a tratamento (GOLFETO & BARBOSA*, 2003*). Estudos que relacionam o TDAH e as comorbidades associadas demonstram que meninas tem mais distúrbios de internalização (depressão e ansiedade), enquanto meninos, distúrbios de externalização (desordens de conduta e opositivas) (HASSON & FINE, 2012; GAUB & CARLSON, 1997; NUSSBAUM, 2011). Tais diferenças podem levar,

na maioria das vezes, ao subdiagnóstico no sexo feminino (BIEDERMAN et al., 1994).

1.1.2 Neurobiologia

Considerando que o TDAH manifesta-se de mais de uma forma, nenhuma alteração em um único sistema de neurotransmissores parece ser responsável pelo aparecimento desse transtorno. Os estudos indicam o envolvimento principalmente das catecolaminas, em especial da dopamina e noradrenalina.

Existem algumas teorias propostas para tentar explicar a neurobiologia do TDAH. A teoria bioquímica se refere quase que exclusivamente à hipofunção do sistema dopaminérgico (CASTELLANOS, 1997). O sistema dopaminérgico executaria ações modulatórias distintas na transferência de informações por meio de circuitos neuronais que conectam o tálamo, o córtex pré-frontal e os neurônios dos gânglios da base (CASTELLANOS, 1997). De maneira geral, as rotas dopaminérgicas mesocorticais (área tegmental ventral e córtex pré-frontal) e nigroestriatal (substância nigra e estriado) estariam predominantemente envolvidas na sintomatologia do TDAH: uma hipofunção nas áreas corticais seria responsável por déficits cognitivos e nas funções executivas, enquanto que uma hiperfuncionalidade dopaminérgica em áreas estriatais resultaria nos sintomas de hiperatividade e impulsividade (THIERRY et al., 1988)

Apesar do papel importante dos sistemas dopaminérgico e noradrenérgico na neurobiologia do TDAH, alguns autores relacionam outros sistemas neurais com a patofisiologia desse transtorno, uma vez que a disfunção dos sistemas dopaminérgicos e noradrenérgicos poderia acarretar disfunções GABAérgicas e glutamatérgicas (POSNER & PETERSON, 1990; PLISZKA, 1992, PLISZKA e cols., 2000). De acordo com essas teorias, projeções excitatórias glutamatérgicas são enviadas do córtex pré-frontal (CPF) para estruturas do estriado, que por sua vez, envia projeções inibitórias GABAérgicas para neurônio da substância nigra. A partir desse evento há inibição dos núcleos talâmicos, que finalizam o circuito com o envio de projeções excitatórias glutamatérgicas para neurônios corticais. Considerando que esses diferentes circuitos estão relacionados com memória, atenção e atividades motoras, explica-se a complexidade e heterogeneidade do TDAH. Um dos autores defende ainda que os vários sintomas do TDAH são resultado de um déficit

do controle inibitório, ou seja, uma falha no controle dos comportamentos e nas funções executivas (BARKLEY, 1997).

A teoria anatomofuncional, descrita por Riesgo e Rohde (2004), descreve disfunções nas áreas frontais e suas conexões subcorticais no sistema límbico. Portanto, no princípio, só havia um sistema atencional e o TDAH era entendido como um fraco controle inibitório frontal sobre as estruturas límbicas. No entanto, a teoria de um único centro atencional - apesar de bem comprovada por estudos neuropsicológicos de neuroimagem funcional e de neurotransmissores - pode explicar alguns casos do TDAH, mas não todos. A visão completa deve incluir uma circuitaria neural com dois sistemas atencionais: o anterior, que envolve a região pré-frontal e suas conexões subcorticais e é responsável pelo controle inibitório e funções executivas, como a memória de trabalho, parece ser dopaminérgico. Já o posterior, parece ser predominantemente noradrenérgico e é responsável pela regulação da atenção seletiva (ROHDE & HALPERN, 2004; RIESGO & ROHDE, 2004). Outra região importante relacionada com atenção é o *locus ceruleus,* o qual é constituído de neurônios adrenérgicos, sendo ativo em respostas a estímulos específicos (PLISZKA et al, 1996).

Ainda, a redução volumétrica de várias regiões cerebrais é bastante relatada, principalmente nas vias ricas em dopamina (DA) na região fronto-estriatal e cerebelo, que também está envolvido na patologia do TDAH (STALLER & FARAONE,2006). Um estudo demonstrou menor volume na região vermal do cerebelo em meninos com o transtorno, quando comparados aos meninos sem TDAH (BERQUIN, GIEDD & JACOBSEN ,1998). Além disso, parece haver uma lateralidade na diminuição de outras regiões, como no lado direito do lobo frontal, globo pálido e caudado. Em meninas com TDAH também foi encontrado um pequeno volume na região posterior- inferior do vermis do cerebelo quando em comparação às meninas saudáveis (CASTELLANOS et al, 2001). Nesse sentido, com relação às diferenças da neurobiologia entre os gêneros, foi relatada uma diferença estrutural do corpo caloso entre meninas e meninos na adolescência, sendo a parte esplênica menor nas meninas e a rostral, menor nos meninos (NUSSBAUM, 2011).

1.1.3 Diferença entre os sexos no TDAH

No sexo feminino é mais frequente o tipo com predomínio de sintomas de desatenção e parece apresentar, conjuntamente com o tipo combinado, uma taxa mais elevada de prejuízo acadêmico (ROHDE, 2000), uma vez que as meninas parecem ser mais prejudicadas no nível intelectual (GAUB & CARLSON, 1997). Diferentemente do sexo feminino, o masculino demonstra pronunciada hiperatividade (POETA & NETO, 2004, GAUB & CARLSON, 1997).

Quando os indivíduos com TDAH passam para a idade adulta, a proporção entre os sexos se inverte, isto é, enquanto nos meninos os sintomas podem estar declinando, nas meninas podem estar aumentando (TAYLOR & KELTNER, 2002). Isto pode ser explicado pela influência do estrogênio no aumento da concentração dos neurotransmissores DA, serotonina e noradrenalina (NE), como visto em um estudo feito por Archer (1999). Fink et al (1996) encontraram um aumento significativo de DA no estriado de fêmeas em resposta à estimulação do estrogênio. Em estudos feitos com ratos e ratas verificou-se que durante o desenvolvimento pré-púbere dos machos há uma superprodução de receptores dopaminérgicos no corpo estriado, o que pode contribuir para a hiperatividade. No entanto, quando eles atingem a fase adulta, existe uma redução de 55% na densidade dos receptores, ao passo que nas fêmeas há um aumento dos receptores dopaminérgico no estriado pela influência do estrogênio (ANDERSEN & TEICHER, 2000).

1.1.4 TDAH e Transtorno por Uso de Substâncias

Cerca de um total de 50% a 90% de todas as crianças com TDAH possuem outras doenças psiquiátricas (SPENCER, BIEDERMAN, & WILENS, 1999; WILENS et al., 2002; NUSSBAUM, 2011) e dentre elas, os transtornos relacionados ao abuso e dependência de álcool e outras drogas (ADAD) estão entre os mais prevalentes (KLASSEN et al, 2012), o que pode intensificar os sintomas do TDAH e dificultar o diagnóstico. Devido a essas alterações, é necessário haver uma história clara de que os sintomas do TDAH são anteriores ao uso de substâncias antes de diagnosticar o transtorno em pacientes que fazem o abuso de drogas (MARIANI & LEVIN, 2007).

Os indivíduos adolescentes com TDAH são duas vezes mais suscetíveis a usar drogas durante a adolescência, sendo que, muitas vezes, buscam essas substâncias com o intuito de acalmar os sentimentos de ansiedade e inquietação consequentes da desordem; já outros procuram os estimulantes para aumentar a capacidade de concentração (“auto-medicação”) (RESTAK, 2003). Em comparação com indivíduos normais controles, pacientes com o TDAH parecem apresentar um consumo mais precoce de álcool e outras drogas, inclusive em termos de quantidades e dependência (VENDRUSCOLO & TAKAHASHI, 2010).

Há um grau de associação relativamente alto entre TDAH e uso de cocaína na adolescência e na idade adulta (BIEDERMAN e cols., 1995; MCGOUGH e cols., 2005). De acordo com Biederman (1995) e Wilens (1994), 50% dos adultos com TDAH têm uma história de abuso de substâncias. Mais recentemente, Wilens e cols. (2004) mostraram que adultos diagnosticados com TDAH apresentam um potencial de risco maior de desenvolver quadros de dependência à cocaína do que os indivíduos sem o transtorno. Ainda, um estudo realizado entre adolescentes em tratamento de dependências de drogas, revelou que 34% deles apresentavam paralelamente o diagnóstico de TDAH (GORDON et al., 2004). Szobot et al.(2007) mostraram, em um estudo realizado com 968 adolescentes brasileiros do sexo masculino, que há uma forte relação entre o TDAH e a probabilidade do desenvolvimento de uso de substâncias. Em 2011, uma meta-análise publicada por Lee e cols com a revisão de mais de 20 estudos, reafirmou que o TDAH na infância está associado a um risco significativamente maior (2x) de uso de cocaína e de outras drogas na fase da adolescência e adultez jovem. Além disso, adolescentes com TDAH, quando comparados com seus irmãos que não apresentam o transtorno, possuem um risco aumentado de desenvolver abuso de substâncias, bem como iniciam o uso mais cedo e de forma crônica (BARKLEY et al., 2004; KING et al.,1999; RIGGS,1998). O interesse desses indivíduos pelo uso de substâncias psicoativas é influenciado pela atração pela novidade e à impulsividade característica (BIEDERMAN et al., 2008; MOLINA,SMITH, & PELHAM, 1999).

Em pacientes diagnosticados com TDAH, apesar da piora acentuada nos quadros de dependência química ocasionado pelo abuso de cocaína, há uma melhora sintomática do TDAH, o que propicia um contínuo re-uso da droga (CASTAÑEDA E cols., 2000). Em vez de excitação, os pacientes com TDAH, sentem efeitos benéficos como diminuição da agitação motora, estabilização do humor,

melhora na atenção e capacidade de pensar (CASTENEDA et al., 1999; CARROLL & ROUNSAVILLE, 1993). Isto acontece, pois há relação entre o mecanismo de ação da droga e a fisiopatologia do transtorno, já que ambas estão diretamente relacionadas à sinalização de DA e NE, assim como ocorre com o metilfenidato, fármaco utilizado no tratamento do transtorno (DEL CAMPO e*t al.,* 2011; VOLKOW *et al*., 2009).

Assim como na clínica, estudos pré-clínicos, como o realizado em nosso laboratório, por Azeredo e cols (2010), mostraram que a cocaína em baixas doses reverte a hiperatividade locomotora em ratos induzidos ao TDAH pelo modelo de lesão dopaminérgica neonatal com 6-Hidroxidopamina (6-OHDA), demonstrando o efeito paradoxal desta droga. Este resultado pode ser explicado pelo aumento da concentração de DA extracelular causada pela administração de cocaína que inverteria a diminuição da funcionalidade da via mesolímbica dopaminérgica lesionada pela neurotoxina 6-OHDA, atenuando, dessa forma, a hiperatividade deste modelo de TDAH (VOLKOW et al., 2002).

1.1.5 Modelos animais de TDAH

O estudo da neurobiologia e sua relação com o comportamento referente ao TDAH pode ser realizado com a utilização de modelos animais. Embora estes animais possuam um sistema nervoso com funcionalidade relativamente simples, o estudo desta patologia psiquiátrica é bem aplicado. Vários modelos animais tem sido usados nas últimas décadas para estudar diversos aspectos do transtorno (VAN DER KOOIJ & GLENNON, 2007): a) modelos de camundongos resultantes de alterações genéticas: camundongo mutante Coloboma, camundongos knock out/down para o gene transportadores de dopamina (DAT), camundongo mutante para o receptor tireoidiano beta; b) modelos de ratos resultantes de alterações genéticas: ratos espontaneamente hipertensos (SHR); Wistar-Kyoto derivado de rato hipertenso (WKHA); c) modelos resultantes de lesões: hipóxia neonatal, 5-bromo-2'deoxiuridina (BrdU) pré-natal, atrofia cerebelar, irradiação do hipocampo e lesão dopaminérgica neonatal . Dentre os modelos, os mais utilizados nas pesquisas pré-clínicas são os modelos SHR e lesão dopaminérgica neonatal.

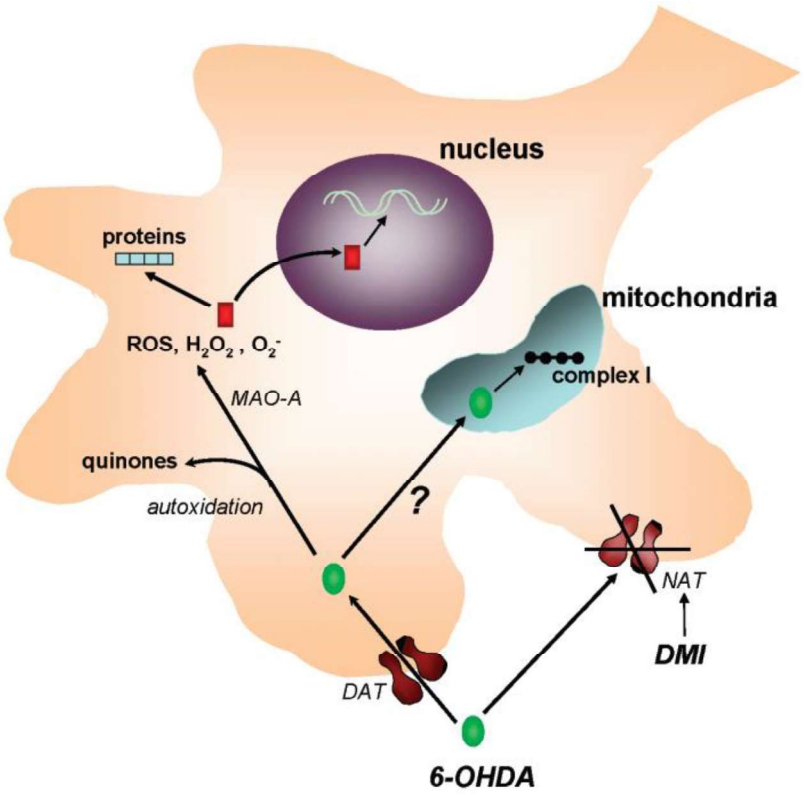
Um modelo animal para ser considerado adequado, precisa preencher três critérios de validade: a validade de face, ou seja, mimetizar os déficits

comportamentais fundamentais que caracterizam a sintomatologia do TDAH; validade preditiva, ou seja, o modelo animal precisa responder aos tratamentos farmacológicos do TDAH; validade de construto, ou seja, o modelo animal deve estar em conformidade com a fundamentação teórica do transtorno (função dopaminérgica alterada) (RUSSELL et al., 2005; SAGVOLDEN, 2000; SAGVOLDEN et al., 2005). Tendo em vista esses critérios, o modelo de ratos espontaneamente hipertensos tem sido questionado, pois estudos mostraram que este modelo falha no que diz respeito à validade preditiva, uma vez que há um aumento da hiperatividade quando estes animais são tratados com metilfenidato e anfetamina, diferentemente do que acontece na clínica (AMINI et al., 2004).

Por outro lado, o modelo de lesão dopaminérgica neonatal por 6-OHDA se enquadra mais completamente nos três critérios de validade, uma vez que acarreta uma hiperatividade motora característica do TDAH (SHAYWITZ et al., 1976) , além de déficit de aprendizagem e de memória (LUTHMAN et al., 1989; ARCHER et al., 1988) alterações dopaminérgicas e diminuição da hiperatividade com a administração de psicoestimulantes, como metilfenidato. Uma revisão de Van Der Kooij & Glennon (2007) concluiu que o modelo de lesão dopaminérgica neonatal é o modelo que melhor representa comportamentalmente e farmacologicamente o TDAH.

A 6-OHDA é uma neurotoxina que ocasiona dano nos terminais catecolaminérgicos através de uma ligação tóxica irreversível aos transportadores destes neurônios (PEARSON et al., 1980). A molécula de 6-OHDA, por apresentar estrutura similar às catecolaminas endógenas (BLUM et al.,2001) é absorvida e armazenada intracelularmente através dos transportadores de membrana dos neurônios dopaminérgicos e noradrenérgicos. Após sua entrada nas células neuronais a neurotoxina pode ser degradada pela monoamina-oxidade (MAO) ou por auto-oxidação, gerando várias espécies citotóxicas, como ROS (espécies reativas de oxigênio), H_2O_2 (peróxido de hidrogênio) e quinonas, que danificam proteínas intracelulares e o núcleo, produzindo dano neuronal. Além desses eventos, a 6-OHDA pode induzir toxicidade neuronal alterando a atividade mitocondrial (Figura 1) (COHEN, 1984; LUTHMAN et al., 1989; VAN KAMPEN et al., 2000, SIMOLA et al., 2007). A fim de selecionar como único alvo os neurônios dopaminérgicos, é administrada intraperitonealmente 30 minutos antes da injeção de 6-OHDA um bloqueador dos transportadores de noradrenalina, a desipramina,

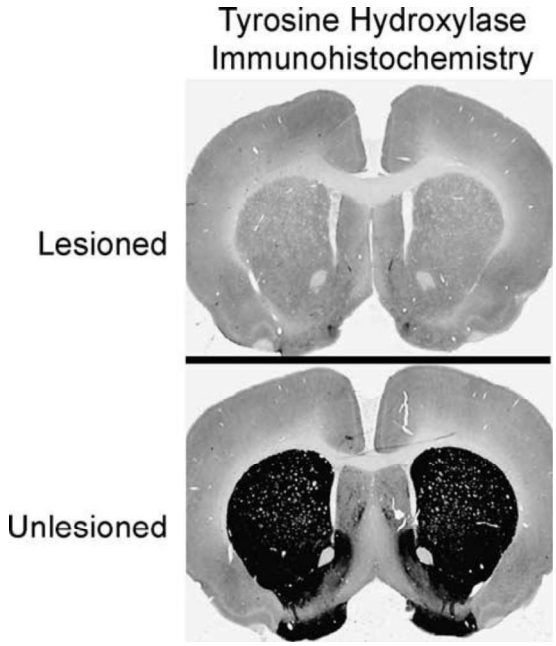
evitando assim, o dano dos terminais noradrenérgicos. Dessa forma, acredita-se que ao lesionar os neurônios dopaminérgicos e proteger os noradrenérgicos, ocorre uma hiperinervação serotoninérgica, acarretando um aumento da serotonina no estriado, que pode contribuir para o aparecimento de comportamentos disfuncionais (MRINI et al., 1995; MOLINA-HOLGADO,1994; KOSTRZEWA, 1998). As lesões ocasionadas nos neurônios dopaminérgicos são geralmente parciais, resultando em cerca de 80% de fibras lesionadas após a injeção intracisternal da neurotoxina (SHAYWITZ e cols., 1976). Na figura 2 é possível observar a imunorreatividade estriatal da tirosina hidroxilase, ilustrando a destruição seletiva dos neurônios dopaminérgicos após lesão neonatal com 6-OHDA.



Fonte: SIMOLA et al., 2007.

Figura 1: Mecanismos de neurotoxicidade induzida por 6-OHDA. Após ser recaptada do espaço extracelular através do DAT ou NAT, a molécula de 6-OHDA é armazenada nos neurônios catecolaminérgicos. Uma vez dentro dos neurônios, a 6-OHDA sofre degradação enzimática pela MAO-A e auto-oxidação, resultando em espécies citotóxicas, as quais, através de danos em proteínas intracelulares e

núcleo, produzem dano neuronal. Além disso, 6-OHDA pode induzir neurotoxicidade por prejudicar a atividade do complexo mitocondrial I. Em experimentos animais, a 6-OHDA é usualmente administrada em associação com bloqueadores da NAT, como a DMI, para prevenir que a molécula seja recaptada pelos terminais noradrenérgicos e para selecionar como alvo os neurônios dopaminérgicos. *DAT*: Transportadores de Dopamina; *NAT*: Transportadores de Noradrenalina; *DMI*: Desipramina; *MAO-A*: Monoamina-oxidase A; *ROS*: Espécies Reativas de Oxigênio; H_2O_2 : Peróxido de Hidrogênio; *6-OHDA*: 6-Hidroxidopamina.



Fonte: BREESE et al., 2005

Figura 2: Imunohistoquímica da tirosina hidroxilase após o tratamento neonatal com 6-hidroxidopamina. Este rato adulto recebeu desipramina 30 min antes da administração intracisternal de 6- OHDA aos 3 dias de idade.

1.1.6 Modelos animais para dependência de drogas.

Inúmeras técnicas foram desenvolvidas para modelar aspectos específicos do comportamento de consumo de drogas, tornando possível a compreensão das bases neurobiológicas e os sistemas cerebrais envolvidos nas propriedades de

recompensa das substâncias psicoativas. No entanto, nas últimas décadas, novos modelos foram propostos na tentativa de desvendar mais especificamente os mecanismos de dependência (VANDERSCHUREN & AHMED, 2013). Os modelos de maior importância são o modelo de livre escolha (*free-choice bottle model* ou *two-bottle choice technique*), o modelo de preferência de lugar (*conditioned place preference*) e o modelo de autoadministração em caixas de condicionamento operante (*operant self-administration*).

O modelo de livre escolha é um método de autoadministração não operante em que o animal tem à disposição duas garrafas, uma com água e outra com a droga em estudo. Dessa forma pode-se verificar a preferência do animal, bem como estimar a dose de droga ingerida. Este modelo é muito utilizado para estudos com álcool (PLANETA, 2013), porém vários estudos com cocaína já foram publicados utilizando esse modelo (MARQUARDT et al., 2004; BARROS & MICZEK,1996, AZEREDO et al.,2010) embora essa via não seja a principal entre os usuários de cocaína. Apesar disso, um estudo demonstrou que a concentração plasmática da cocaína em humanos tem o mesmo perfil de concentração após o uso pela via intravenosa (FANG et al., 1999). Dois estudos realizados em nosso laboratório verificaram que a administração oral de cocaína através deste modelo é capaz de induzir reações à retirada da droga, como o aumento de vocalizações ultrassônicas (BARROS & MICZEK,1996) e comportamento tipo-depressivo no teste de nado forçado (MARQUARDT et al., 2004).

O modelo de preferência de lugar é utilizado em animais de laboratório para medir a recompensa de drogas. Neste método são usadas caixas que possuem três compartimentos: dois espaços com características diferentes (por exemplo, com cores e materiais diferentes) e um espaço neutro. Os animais são condicionados a associar os efeitos da droga a um dos compartimentos. O experimento consiste em primeiramente ambientar o animal às diferentes câmeras; na próxima fase administra-se a droga em estudo e coloca-se o animal confinado em um dos compartimentos; e na última fase coloca-se o animal no espaço neutro e verifica-se para qual dos compartimentos ele vai se direcionar. Dessa forma é possível analisar o potencial de reforço da droga se o animal passar a maior parte do tempo no compartimento em que aprendeu a associar à substância (BARDO & BEVINS, 2000; CRUZ et al.,2010).

O modelo de autoadministração em caixas de condicionamento operante é o que melhor avalia as propriedades reforçadoras de uma droga, uma vez que testa o quanto o animal trabalha para receber a substância (PLANETA, 2013). As caixas contem barras que ao serem pressionadas acionam uma bomba externa contendo a solução a ser injetada (Figura 3). Esse sistema é conectado a um computador que contém um programa específico com o protocolo de interesse programado pelo pesquisador. A primeira fase deste modelo consiste em um treinamento para aquisição do comportamento operante, ou seja, fase em que os animais aprendem que a pressão na barra gera a liberação de um reforço, que pode ser realizada com alimento ou solução com sacarose, dependendo do modelo de caixa utilizada. Após a fase de aprendizado, inicia a fase do teste onde o reforço do treino é substituído pela solução com a droga a ser estudada, sendo que o reforço pode ser via oral ou intravenosa (PLANETA, 2013; FRANS VAN HAAREN, 1993).

Figura 3: Sistema de autoadministração (registros do autor). Composto por caixa de autoadministração contendo duas alavancas em uma das paredes, que acionam a bomba de infusão após pressionadas. Entre as alavancas há um orifício por onde o animal consome a solução oral. As caixas de autoadminitração são envoltas por um caixa de madeira, para evitar grande propagação de ruído para a sala de experimentação.

Em experimentos com animais, já está bem estabelecido que existem diferenças entre machos e fêmeas na resposta comportamental a psicoestimulantes (PERROTI et al., 2001; QUINONES-JENAB et al., 1999). Ratas fêmeas apresentam maior consumo de cocaína durante a sessão de autoadministração e aumento de resposta a esquemas de razão progressiva de reforço (KERSTETTER AND KIPPIN, 2011). As fêmeas também mostram maior resposta locomotora e maior sensibilização a psicoestimulantes (WISSMAN et al., 2011).

Em um estudo realizado por Russo e cols (2003) para determinar a preferência condicionada de lugar por diferentes doses de cocaína em ratos de ambos os sexos, verificou-se que as fêmeas preferiam as doses mais baixas (5 e 10 mg/kg) e os machos a dose elevada (20mg/kg).

Roth & Carroll (2004) compararam a autoadministração intravenosa entre machos e fêmeas e verificaram que as fêmeas se autoadministram mais que os machos em sessões longas de 6 horas de duração. Já quando a sessão tinha duração de 1 hora, os machos apresentavam maior número de reforços que as fêmeas.

1.2 SISTEMA GABAÉRGICO

1.2.1 Ácido γ-Aminobutírico (GABA)

O ácido gama – aminobutírico (GABA) é considerado o mais importante neurotransmissor inibitório do SNC (TWYMAN & MACDONALD, 1991) e como tal, desempenha um papel fundamental na modulação da atividade neuronal (BETTLER et al., 2004), tanto em termos de excitações ou inibições sinápticas, interagindo com outros sistemas neuronais, como os sistemas dopaminérgico, noradrenérgico e glutamatérgico, regulando de forma específica as interações entre neurônios adjacentes em distintas áreas do SNC [GRAY e cols., 1991].

O sistema GABA é um dos que nos interessa estudar na relação de desenvolvimento de dependência aos psicoestimulantes, pois estudos recentes mostram modulação deste sistema pelo uso continuado de cocaína (AZEREDO e cols., 2010). Estas alterações de plasticidade estão citadas como mecanismos importantes da dependência aos psicoestimulantes, e o sistema GABA tem sido apontado como um potencial alvo para o tratamento deste transtorno psiquiátrico. Nas últimas décadas estão se acumulando evidências que o GABA tem participação nos efeitos da cocaína. Em estudo realizado em nosso laboratório, a autoadministração de cocaína provoca mudanças na transcrição gênica de diversas subunidades do receptor $GABA_A$ no córtex pré-frontal, hipocampo e estriado de ratos no modelo animal de lesão dopaminérgica neonatal (AZEREDO e cols., 2010).

O neurotransmissor GABA é sintetizado a partir do glutamato como produto de uma reação catalisada pelas enzimas ácido-glutâmico descarboxilases (GAD_{65} e

GAD_{67}) encontradas apenas em neurônios que sintetizam GABA no cérebro (ERLANDER, et al., 1991), e então é carregado por vesículas transportadoras de neurotransmissores e liberadas dos terminais nervosos por exocitose dependente de cálcio (FON & EDWARDS, 2001). Após sua ação, o GABA é metabolizado a succinato através de duas reações pelas enzimas GABA-transaminase e semialdeído-succinato desidrogenase [KORPI, 2002]. A enzima GABA-transaminase ocorre em neurônios pós-sinápticos e em células da glia, onde participa da inativação do GABA após sua liberação, sendo por isso considerado responsável pelo controle da manutenção de concentrações apropriadas de GABA pré-sináptico (DELUCIA et al., 2007)

O GABA é particularmente abundante no cérebro. Nos sistemas neuronais como os localizados nos gânglios basais, especialmente a substância negra e o globo pálido concentram altos níveis de GABA (cerca de 10μmol/g de tecido), o que sugere a importância desse neurotransmissor nessa área cerebral. Já na substância cinzenta o GABA ocorre em concentrações mais baixas (2-5μmol/g de tecido). O conteúdo do GABA é de 200 a 1000 vezes maior do que de outros neurotransmissores. No tecido estriatal, o GABA é o principal transmissor e está localizado nos interneurônios curtos e em neurônios que se projetam para a substância negra e o globo pálido. Praticamente todos os neurônios são sensíveis ao efeito inibitório do GABA, ou seja, ele se distribui por todo o sistema nervoso central (SNC), demonstrando ter uma ação em quase todas as áreas cerebrais ao mesmo tempo (RANG, 2004; DELUCIA et al., 2007).

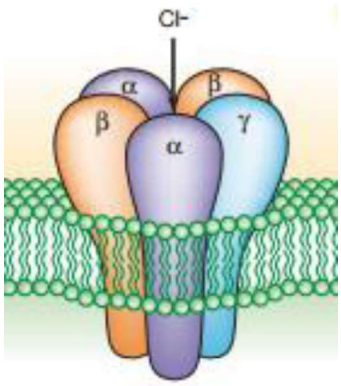
1.2.2 Receptores $GABA_A$

Os efeitos do neurotransmissor GABA são mediados através da ativação de receptores ionotrópicos e metabotrópicos: $GABA_A$, $GABA_B$, $GABA_C$ localizados nos neurônios pré ou pós-sinápticos (CHERUBINI & CONTI, 2001) . Os receptores $GABA_A$ e $GABA_C$ são ionotrópicos, ou seja, consistem em um canal iônico regulado por ligante, enquanto o $GABA_B$ é metabotrópico, acoplado à proteína G. Os receptores $GABA_C$ são uma variante dos receptores $GABA_A$ diferenciados na retina (OLSEN & SIEGHART, 2009).

Os receptores $GABA_A$, de localização pré e pós-sináptica, medeiam a maioria das neurotransmissões inibitórias rápidas no cérebro de mamíferos. A ligação do

ligante neste receptor causa uma mudança conformacional na proteína do canal que resulta em influxo de íons através dos poros para o interior da célula. Esses íons, principalmente, são os íons Cl^-, no entanto o ânion HCO_3^- (bicarbonato) também pode permear o poro do canal, ainda que de forma menos eficiente. Como o potencial de membrana do Cl^- é, em geral, negativo em relação ao potencial de repouso da célula, o aumento da permeabilidade deste íon hiperpolariza a célula, reduzindo, assim, a sua excitabilidade (OWENS & KRIEGSTEIN 2002; RANG, 2004; KAILA, 1994; BORMANN, et al., 1987).

A composição proteica do $GABA_A$ é apresentada na forma de um complexo glicoproteico transmembrânico heteropentamérico, ou seja, a estrutura é composta por cinco subunidades polipeptídicas, sendo que o arranjo conformacional dessas subunidades define sua funcionalidade dentro de um circuito neuronal (Figura 4). Já foram identificadas seis subunidades α, três β, três γ, uma δ, uma ε, uma π e uma θ, totalizando 16 subunidades que são codificadas a partir de 19 genes (BORMANN, et al., 1987; MACDONALD & OLSEN, 1994; MEHTA & TICKU, 1999, SIMON et al., 2004). No SNC, este receptor pode possuir diversas combinações dessas subunidades, podendo resultar em alterações na afinidade dos ligantes pelos receptores, e com isso, resultar em respostas diferentes a determinados ligantes (BARNARD et al., 1998). Está descrito que em ratos as principais subunidades proteicas que compõem os receptores $GABA_A$ são α, β, γ e δ, e as combinações apresentadas são formadas pelas subunidades $\alpha 1\beta\xi\gamma 2$, $\alpha 2\beta\xi\gamma 2$, $\alpha 3\beta\xi\gamma 2$, $\alpha 4\beta\xi\gamma 2$, $\alpha 5\beta\xi\gamma 2$, $\alpha 6\beta\xi\gamma 2$, $\alpha 4\beta\xi\delta$ e $\alpha 6\beta\xi\delta$ (JECHLINGER et al., 1998; OLSEN & SIEGHART, 2009). Além disso, foi verificado em neurônios de ratos a diferenciação em relação às combinações de subunidades dependendo da estrutura cerebral (BARNARD et al., 1998).



Fonte: BELELLI & LAMBERT, 2005

Figura 4: Representação do receptor $GABA_A$ formado por um complexo glicoproteico transmembrânico

1.2.3 Sistema GABAérgico e 6-OHDA

Estudos já realizados demonstram que alterações no sistema dopaminérgico acarretam mudanças no sistema GABAérgico de ratos lesionados por 6-OHDA. Chadha et. al. (2000) mostrou, após lesão dopaminérgica no trato nigroestriatal de ratos, que ocorre uma alteração na expressão gênica das subunidades $\alpha1$, $\alpha4$, $\beta2$ e $\gamma2$ do receptor $GABA_A$ no tálamo e gânglio basal. As alterações mais relevantes encontradas foram um aumento da expressão das subunidades $\alpha1$ e $\beta2$ na região nigroestriatal, e uma paralela redução dessas subunidades no globo pálido. Estudo realizado em nosso laboratório em ratos com lesão dopaminérgica por 6-OHDA via intracisternal, verificou aumento da expressão de RNA mensageiro da subunidade $\alpha2$, $\alpha4$ e $\beta2$ no córtex pré-frontal, aumento da expressão das subunidades $\beta1$ e $\beta2$ no hipocampo, e no estriado foi encontrado um aumento da expressão de $\alpha2$ e uma diminuição na expressão de $\beta2$ (AZEREDO et al., 2010).

Embora tenha realizado a lesão dopaminérgica pela via subcutânea em ratos recém-nascidos, cujo mecanismo é provavelmente diferente, o estudo realizado por Podkletnova et al (2000) é de grande relevância, pois mostrou uma redução significativa dos níveis de expressão de RNA mensageiro das subunidades $\alpha1$, $\alpha6$ e $\gamma2$ de $GABA_A$ no neocerebelo e um atraso temporário adicional na transcrição pós-natal de subunidades $\alpha1$ e $\gamma2$ no córtex pré-frontal.

1.2.4 Sistema GABAérgico e Cocaína

Após o uso de cocaína ocorre a liberação de DA no núcleo accumbens e estriado liberados das projeções dopaminérgicas da substância nigra e da área tegmental ventral (KALIVAS et al., 1990). Vários estudos sugerem que o sistema GABAérgico pode alterar a atividade dessas projeções dopaminérgicas (KALIVAS et al., 1990; STEIN, 1998; YANG e MOGENSON, 1985, 1989). Devido à estreita interação entre esses dois sistemas, os receptores $GABA_A$ podem ser afetados pela cocaína, os quais podem participar das alterações cerebrais causadas pela liberação de dopamina (YAMAGUCHI, 2000). Embora isso seja já de conhecimento da comunidade científica, o mecanismo através do qual a cocaína interage com os neurônios GABAérgicos ainda não está bem elucidado (KALIVAS,2007; ARIANO, 1996). Estudos mostraram que repetidas injeções de cocaína diminui o número de receptores $GABA_A$ e a estimulação do $GABA_A$ pela absorção de Cl^- no estriado de ratos (PECINS-THOMPSON & PERIS, 1993; PERIS, 1996). Outro estudo verificou que em ratos sensibilizados à cocaína há uma diminuição da expressão da subunidade α2 no núcleo accumbens (CHEN et al., 2007).

Yamaguchi (2000) demonstrou que após 30 minutos de uma injeção aguda de cocaína há uma diminuição da expressão da subunidade α1 no córtex parietal e cerebelo de ratos, porém essa diminuição é revertida no cerebelo após 4 horas da injeção. Neste estudo eles também verificaram que, diferentemente do que acontece com α1, a subunidade α6 não apresentou alterações significantes em nenhuma das regiões analisadas, a saber: córtex cingulado, córtex fronto-parietal, córtex temporal, estriado, tálamo, giro dentado e cerebelo. Além dessas alterações, eles observaram que houve redução significante da subunidade β2 no córtex cingulado 30 min depois da injeção, e ainda que β3 diminuiu sua expressão de RNA mensageiro no estriado e um aumento no giro dentado 1 hora depois da injeção. Dessa forma é possível perceber que há uma rápida alteração dos níveis de RNA mensageiro das subunidades do $GABA_A$, demonstrando a ação da cocaína na transcrição ou na degradação do RNAm, ou seja, a cocaína pode ter uma influência muito importante na inibição ou ativação dos fatores de transcrição. Interessantemente, dois estudos apontam que a ativação de receptores de dopamina resulta em aumento da liberação de GABA no córtex (BOURDELAIS & DEUTCH, 1994; GROBIN & DEUTCH, 1998). Sendo assim, as reduções de expressão de RNAm da subunidade

$\alpha 1$ obervada no córtex pode ser explicado por este mecanismo mediado pelo receptor induzido pelo agonista, no caso o GABA (YAMAGUCHI, 2000).

1.2.5 Sistema GABAérgico, 6-OHDA e Cocaína

Estudo realizado em nosso laboratório em animais machos lesionados pela injeção intracisternal por 6-OHDA que foram submetidos à autoadministração oral de cocaína pelo modelo *Two Bottle Choice* demonstrou que a cocaína atenua a expressão de RNAm da subunidade $\alpha 2$ do receptor $GABA_A$ do córtex pré-frontal dos ratos, bem como reverteu o aumento de $\alpha 4$ quando comparados aos ratos lesionados para os quais foi disponibilizado somente água. Quando a expressão de RNAm de $\beta 1$ foi analisada, verificou-se um aumento significante desta subunidade nos ratos lesionados que consumiram cocaína, ao passo que a subunidade $\beta 2$ teve sua expressão atenuada após o consumo oral de cocaína, quando considerado o córtex pré-frontal dos ratos (AZEREDO et al.,2010).

Ao analisar as modificações das subunidades de $GABA_A$ no hipocampo desses animais, verificou-se que as alterações de $\alpha 2$ e $\beta 2$ ocorreram de maneira diferente quando em comparação ao córtex pré-frontal: houve aumento da expressão de RNAm de $\alpha 2$ e expressão maior de $\beta 2$ quando comparado aos controles, sendo que a cocaína não reverteu esse efeito. Por outro lado, houve reversão do aumento da expressão de RNAm da subunidade $\alpha 4$ causada pela 6-OHDA, assim como no córtex, e uma expressão elevada dos animais 6-OHDA de $\beta 1$ em relação aos controles, que também não foi revertida com a autoadministração oral de cocaína (AZEREDO et al.,2010).

Analisou-se também o estriado desses ratos, onde foi detectada uma reversão da expressão de $\alpha 2$ nos animais lesionados que consumiram cocaína. Já em relação às subunidades $\alpha 4$, $\beta 1$ e $\beta 2$ não se detectou alteração na expressão gênica (AZEREDO et al.,2010).

2 JUSTIFICATIVA

O TDAH apresenta-se de maneiras diferentes entre homens e mulheres, e a literatura sugere que indivíduos com TDAH exibem um risco aumentado para o uso

de substância. (BRADY & SINHA, 2005; WILENS et al., 1994). E dessa forma, o uso de substâncias também parece ser diferente entre os sexos nos indivíduos com o transtorno (DISNEY, et al., 1999; BIEDERMAN et al., 2002; STALLER & FARAONE, 2006).

Estudos sugerem que normalmente mulheres são mais suscetíveis aos efeitos das drogas e enfrentam um padrão mais grave de dependência quando comparadas ao sexo masculino (LYNCH et al, 2002; CARROL *et al., 2004;* HU E BECKER, 2008; KERSTETTER & KIPPIN, 2011)**.** Em modelos animais, ratas adquirem comportamento de auto-administração à cocaína mais rapidamente e se auto-administram mais cocaína quando comparadas aos machos (HU et al., 2004; CAMPBELL et al., 2002).

Estudos demonstram que a sintomatologia do Transtorno pode ser amenizada com o uso de drogas, principalmente psicoestimulantes, como a cocaína, que poderia fazer com que indivíduo buscasse no uso uma forma de automedicação. Em animais foi demonstrado que a autoadministração de cocaína em baixas doses reduz a hiperatividade em ratos machos induzidos ao TDAH.

Portanto, com relação ao uso de substância em indivíduos com TDAH, homens e mulheres não podem ser vistos da mesma maneira. Poucos estudos pré-clínicos estudam essas diferenças, principalmente com o modelo de lesão neonatal por 6-OHDA e a autoadministração de psicoestimulantes. A escassa literatura acerca do tema evidencia a importância de mais estudos relacionando diferenças sexuais no TDAH e no abuso de substâncias, para, assim, auxiliar no desenvolvimento de diagnóstico e tratamento destas patologias, uma vez que pode haver diferenças também na eficácia do tratamento.

3 OBJETIVOS

3.1 Objetivo Geral

Verificar os efeitos comportamentais e GABAérgicos da autoadministração oral de cocaína em ratos machos e fêmeas induzidos ao Transtorno de Déficit de Atenção e Hiperatividade por 6-OHDA.

3.2 Objetivos Específicos

-Comparar o comportamento de autoadministração oral de cocaina entre machos e fêmeas lesionados e não lesionados com 6-OHDA

- Verificar os efeitos após prolongada autoadministração de cocaína na hiperatividade de ratos machos e fêmeas.

- Analisar as subunidades α1, α2, e γ2 dos receptores $GABA_A$ no córtex pré-frontal de ratos machos e fêmeas lesionados por 6-OHDA com prolongada autoadministração de cocaína;

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5 ARTIGO CIENTÍFICO

REVISTA: Behavioural Brain Research

Behavioral and GABAergics effects of oral cocaine self-administration in males and females animals with ADHD.

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Abstract

Attention-deficit/hyperactivity disorder frequently co-occurs with substance use disorder (SUD), that is related to altered in the GABAergic pathways. The gender differences influence the symptoms ADHD and substance use. The aim of this study was to investigate the differences between males and females rats in ADHD model on the oral cocaine self-administration and its effects in mRNA expression of $\alpha 1$, $\alpha 2$ e $\gamma 2$ subunits in the prefrontal cortex. ADHD was induced by 6-OHDA intracisternal lesion and the locomotion was evaluated in a locomotor activity test. Oral cocaine solution was offered for 27 days, using the operant self-administration, with different concentrations (0.2mg/ml, 0.3mg/ml and 0.4mg/ml) of cocaine. M

Sham, M 6-OHDA and F Sham increase locomotor after repetead cocaine. 6-OHDA rats had lower reinforcement than sham. M 6-OHDA has lower reinforcement than M S*ham* in all concentrations of cocaine. F 6-OHDA had lower reinforcement than F *Sham*, but it was not significant. F 6-OHDA decreased the reinforcement in higher concentrations of cocaine. The daily dose of cocaine was higher in F *Sham* and this was lower in the F 6-OHDA when increase the cocaine concentration. In these moments M S*ham* and M 6-OHDA had similar daily doses of cocaine. On general, the groups increased the doses with increment of cocaine concentration. The *Sham* and 6-OHDA groups did not show differences in $\alpha 1$, $\alpha 2$ and $\gamma 2$ subunits $GABA_A$ mRNA expression, but we observed direct correlation between three subunits in F *Sham* group and direct correlation between $\alpha 2$ and reinforcement in M 6-OHDA. Our results provide important information about the cocaine reinforcement patterns in animals induced the disorder and the implication of sex in these standards. The paradoxical effect of cocaine does not happen when there is a high consumption of the ADHD animals. The expression of the $GABA_A$ receptor subunits, our findings indicate that there may be significant changes caused by cocaine in animals with ADHD. So we see the need for more studies to verify the consumption of cocaine in male animals and induced ADHD females and changes in $GABA_A$ subunit expression related addition to psychostimulants to elucidate the behavioral and neurochemical bases of the coexistence of these two disorders.

Keywords: ADHD, cocaine self-administration, sex differences, $GABA_A$, Prefrontal cortex

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a heterogeneous develop-mental disorder characterized by hyperactivity, inattention and impulsivity [1]. The prevalence of ADHD in the population is estimated at 5% in children and 2.5% in adults [1]. This disorder is the result of a possible brain abnormality with malfunctions in the catecholaminergic system, especially involving dopamine and norepinephrine [15].

There are sex differences in the ADHD symptoms [5,3]. Specifically, boys are likely to be more hyperactive and impulsive and to have more comorbid externalizing disorders (e.g., conduct disorder, oppositional defiant disorder), whereas girls are

more likely to be inattentive and to have comorbid internalizing disorders (e.g., anxiety, depression) [6,18].

Animal models are required for the study the nature and basis of these differences [3]. Neonatal dopaminergic lesion by 6-hydroxydopamine (6-OHDA) is the first model recognized to induce hyperactivity in rats [24]. The intracisternal injection of 6-OHDA is toxic to both dopaminergic and noradrenergic neurons and consequently reduces DA/NA neurotransmission in the brain. It is also known that if a ADHD model with only DA deficits is warranted, the NA neurons may be protected by pretreatment with a NA reuptake inhibitor. Behavioural consequences of intracisternal 6-OHDA administration to neonatal rat pups include hyperactivity as well as learning and memory deficits [24,25]. A study from our laboratory showed that hyperactivity may last until adulthood [7]. The lesions are associated with behavioral sex differences. Prefrontal 6-OHDA lesion increased locomotor activity in males, but not in females [26].

Adult ADHD is diagnosed in about a quarter of the patients with substance use dependence [27]. Children with ADHD have an elevated risk of becoming abusers of drugs; mainly of cocaine. One of the explanations for the assotiation is because of the paradoxical effects of psichostimulants which reduce symptoms of the disorder in adolescence and adulthood [28,29]. The paradoxical effect of cocaine may be replicated in the ADHD rats, as seen by a decrease in locomotion when the 6-OHDA animals are offered cocaine in a two-bottle choice self-administration method [2].

Non-ADHD human and animal studies show that drugs of abuse affect males and females differently [30]. Sex differences in the profile of cocaine dependence, for example, have indicated that women, relative to men, transition faster from first use to entering treatment, which may be explained by women abusing cocaine more addictive routes more quickly and reporting a more rapid progression of drug dependence [21,30, 8, 16]. Likewise, as children with ADHD grow, the risk of substance use is higher in females than in males [4]. In animals, studies with rats show that females acquire self-administration and develop conditioned place preference to cocaine in fewer sessions than males [10, 12]. Females also show a greater locomotor response and stronger sensitization to repeated cocaine exposure [17, 31].

Several authors relate the pathophysiology of ADHD with other neural systems, as dysfunction in dopaminergic and noradrenergic systems could result in GABAergic and glutamatergic dysfunction [32, 22, 23]. The hypofunctioning mesolimbic,

mesocortical and nigrostriatal dopamine pathways fail to modulate GABAergic signal transmission appropriately [33, 13, 14]. The dopaminergic lesion results in mRNA expression of diverse $GABA_A$ subunits in the prefrontal cortex (PFC), hippocampus and striatum [2]. It is also known that cocaine changes the release of GABA in the ventral tegmental area (VTA) and alters extracellular GABA levels in the prefrontal cortex after subacute exposure to cocaine [34, 35].

In the clinical setting it is thought that females and individuals with ADHD present a greater risk of cocaine abuse. There is few in neurobiological studies about the sex differences in regard to ADHD and SUD and the possible implications of the GABAergic system and there is lack of information regarding the comparison of cocaine self-administration of males and females in animal models of ADHD. Thus, the aim of this study was to verify the effects of oral cocaine self-administration on behaviors of male and female rats lesioned with 6-OHDA and the correspondent association with $GABA_A$ receptors subunits mRNA in the prefrontal cortex.

Materials and methods

Animals

Nineteen female (± 170g) and nineteen male (± 250g) Wistar rats were obtained from the animal house of the Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil (UFCSPA). Rats were housed in groups of five in polypropylene cages (40 × 33 × 17 cm) under standard environmental conditions, such as temperature-controlled rooms (22±2 ◦C) and a 12-h light/dark cycle (7:00 am to 7:00 pm). All animals received rodent chow and filtered drinking water ad libitum until the beginning of self-administration training. During the self-administration procedures water was restricted 19 hours prior to the sessions.

All in vivo experiments followed the guidelines of the International Council for Laboratory Animal Science (ICLAS) and in concordance with the Brazilian law for the Scientific Use of Animals. This study protocol was approved by the Ethical Committee for Animal Experimental Procedures of UFCSPA, Porto Alegre, Brazil (nº221/13).

Drugs

Cocaine hydrochloride (0,2mg/ml; 0,3mg/ml; 0,4mg/ml - Merck, Alemanha) was diluted in a sucrose solution (1.5%). Desipramine HCl, 6-hydroxydopamine (6-

OHDA.HBr), and ascorbic acid (vehicle) were obtained from Sigma Chemical Co. (St Louis, Missouri, USA). Desipramine was diluted in saline (2mg/ml). A solution containing 6-OHDA (0.1mg/ml) dissolved in Ringer solution containing 0.1 mg/ml ascorbic acid vehicle was administered intracisternally (i.c.).

Procedures

Induction ADHD by lesion 6-OHDA

For each dam, pups were randomly assigned to receive 6-OHDA and others to receive the control solution, according to the sex. On PND4, before receiving lesions, 6-OHDA pups were given intraperitoneal injections of desipramine hydrochloride (20 mg/kg body weight, in 0.1mL) and sham pups were given intraperitoneal saline. After 30 min, sham animals received a 10µL (Hamilton syringe) intracisternal injection of vehicle containing 0.1% (w/v) ascorbic acid and the lesioned group, received the same volume of 6-OHDA solution (100 µg free base), into the cisternal space, with their heads positioned in hiperflexion to expose the suboccipital space. The animals were then returned to their mothers. After 21 days, the pups were weaned. In an earlier study, this procedure consistently produced a 98–99% depletion of dopamine, and desipramine effectively protected norepinephrine neurons from the destructive action of 6-OHDA [24].

Self administration

Apparatus

Experiments in rats were conducted in standard experimental chambers (25x37x28 cm high) made of acrylic and metal with stainless steel rod floor. Each chamber was equipped with two levers located on the same wall, in which only one of them was active. The experimental chambers were placed inside of sound proof timber chambers. The experimental chamber was connected to an infusion pump containing a syringe to infuse the drug solution with appropriate rate of infusion (Insight, Brazil). The pressure in the active lever releases 0.2 ml of solution per 10 seconds. Lever pressure during infusion did not produce a second injection, but lever pressure immediately after completion of the infusion produced another injection. Responses on the inactive lever were recorded, but had no consequences. The computer with the Insight® software controlled the program and recorded data.

Self-administration training sucrose

During 7 day lesioned and sham rats (PND= 50) were trained to press the active lever (left or right, counterbalanced across rats) on a fixed-ratio 1(FR1) schedule for sucrose solution (15.10^{-2}/ml) in operant chambers. The sucrose solution was released on a dispenser. Sucrose solution training sessions lasted for 3 hours. The training sessions ended when the animal was able to hit 85% or higher in the active lever in three consecutive daily sessions. In this training the rats were overnight with water restriction. The three hour sessions were conducted daily Monday–Saturday.

Cocaine Self-administration

After the training sessions the animals were assigned to the cocaine self-administration sessions, for 27 days, also on a FR1 schedule, lasting for 3 hours or until rats received 200 drug infusions. During the first 9 days, each active lever press resulted in cocaine 0,2mg/ml infusion. The concentration of the cocaine solution was changed to 0.3 mg / ml in the tenth day of session, and to 0.4 mg / ml on the nineteenth day session until the 27th session.

Behaviour analysis

Locomotor Activity

Locomotor Activity was used to measure the spontaneous activity of 6-OHDA and *sham* rats. Each automated device, consisting of rodent test chambers (80 x 26 x 22 cm; Alsbarch, Porto Alegre, BR) each placed within 4 series of infra-red beams. It was registered by the low grid of infra-red beams. The photocell cage was coupled to a digital counter. Each rat was allowed to remain in the test chamber for 20 min, of which the first 5 min period were considered ambiance [9].

We evaluated the behavior of animals in the locomotion chamber before beginning of self-administration (basal-PND 45-50) and 26th of cocaine to verify if there was an alteration of hyperactive behaviors in male and female rats, as observed in oral cocaine intake reduces hyperactivity in male rats [2].

Real-time quantitative PCR (qPCR)

Reverse transcription combined with real-time quantitative PCR (qPCR) and the 2− ΔΔCT method was used to determine relative gene expression of $GABA_AR$ γ_2, α_1 e α_2 subunit in the prefrontal cortex. Total RNA was extracted from the prefrontal cortex using the TrizolTM Isolation Reagent Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions, and samples were stored at −80 ◦C. cDNA was synthesized using SuperScript III (Life Technologies, Carlsbad, CA, USA). DNA was quantified by using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). qPCR analysis was performed at least in duplicate using the StepOnePlus Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). The set of primers for $GABA_A$ γ_2 subunit (sense: 5 - GACGATGACCACTCTCAGCA-3 and antisense: 5 - ACAGTCCTTGCCATCCAAAC-3), α_2 subunit (sense: 5 - GACAATGACCACATTAAGCATCAG- 3 and antisense: 5 - GAGAACAAGCCAGCCGAAGCCAAGA-3), α_1 subunit (sense: 5 - AAGGACCCATGACAGTGCTC-3 and antisense: 5 - GGCTCCCTTGTCCACTCATA-3), and β-actin (endogenous control) (sense: 5 - TGTGATGGTGGGAATGGGTCAG-3 and antisense: 5 - TTTGATGTCACGCACGATTTCC-3) was chosen from *Rattus norvegicus* data from the National Center for Biotechnology Information. The reaction contained 50 ng cDNA, 2× Power SYBR Green PCR Master Mix (Life Technologies, Carlsbad, CA, USA), and 0.17μM of each primer. The genes were amplified with an initial denaturation at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min and were succeeded by the melting curve stage.

We performed this analysis to know if there was any correlation between the behavior and the $GABA_A$ subunits mRNA levels.

Statistical analyses

For the analysis of locomotion activity basal, we used a Two Way ANOVA, considering factors lesion/sex and day. For the analysis of cocaine effects on locomotion behavior, we used a Two Way RM ANOVA, considering factors cocaine treatment days (baseline, cocaine day 26) and sex (female or male). For the analysis of

self-administration parameters, we used a Two Way ANOVA, considering factors sex (female or male) and lesion/solution concentration. For the analysis of self-administration parameters, we used a Two Way RM ANOVA, considering factors sex (female or male), day (1 to 27) and lesion. The Tukey test was used for post hoc comparisons when appropriate. For the analyses of *qPCR* was used Two Way ANOVA, with sex and lesion as factors. For the analyses of correlation between parameters was used Pearson Correlation. All data were presented as means ± standard error of the mean. Differences were considered significant at $P < 0.05$. The Sigma Stat program (Jandel Scientific Co., v. 3.2, San Jose, USA) was used.

Results

Effects 6-OHDA lesion on locomotor activity

Our results showed that the neonatal dopaminergic lesion increased the overall locomotion in the 6-OHDA animals ($F_{(1,34)} = 5.901$, $P = 0.021$) (Fig.1). It is also seen that the locomotor activity of the female rats was higher than the male rats ($F_{(1,34)} = 5.901$, $P = 0.021$) maily because hyperlocomotion due to 6-OHDA is seen in male rats, while *Sham* males have lower locomotor activity than *Sham* females (Fig.1).

Effects of cocaine self-administration on locomotor activity

As showed in Fig.2, we found differences between the locomotion days ($F_{(1, 27)} = 35.05$, $P < 0.001$) and interaction between group vs. day of locomotion ($F_{(3, 27)} = 3$, $P = 0.048$). We detected an increased of locomotor activity level on 26th day, when compared to baseline day within M 6-OHDA group ($P = 0.016$). In the F 6-OHDA group we not found differences between days. The locomotion on the 26th days was higher than of baseline day within M *Sham* group ($P< 0.001$). The female rats sham also had increase of locomotion on the 26th of test ($P = 0.019$).

Oral Cocaine self-administration

The Fig.3 shows the daily cocaine reinforcement mean in 27 days. The reinforcement mean was higher in the sham rats than of the 6-OHDA rats ($F_{(1, 34)} =$

6.81, *P* = 0.013).

Significant interaction was detected between groups with concentration of cocaine ($F_{(9,1.221)}$ = 5.41, *P* < 0.001). These results are shown in Fig. 4. The reinforcement of M 6-OHDA group was lower than M *Sham* in all concentrations (P < 0.001). F *Sham* group had lower reinforcement than M *Sham* in 0 and 0.2 concentration of cocaine (P < 0.001). F 6-OHDA showed an increase of reinforcement in concentration 0 cocaine when compared to F *Sham* (P = 0.003). The reinforcement number was higher in F 6-OHDA than the M 6-OHDA in concentrations 0, 0.3 and 0.4 (P < 0.05). The M *Sham* group presented a decrease of reinforcement in the concentration of 0.3 and 0.4 when compared to 0.2 (P < 0.001) and a decrease in the concentration 0.4 when compared to 0 (P < 0.05). In the F 6-OHDA group the reinforcement number decreased too when compared the 0 concentration to 0.3 and 0.4 (P<0.05). There was no difference in the M 6-OHDA and F *Sham* groups between the concentrations.

Our results showed a significant interaction between group and day when we analyzed the cocaine intake between day 1 and day 27 ($F_{(78,571)}$ = 1.791, P = 0.035). Within the M *Sham,* M 6-OHDA and F 6-OHDA groups there are no difference doses between days. Only F *Sham* group show doses differences between days: on the day 23 the cocaine intake was higher than day 1, 2, 3, 4, 6, 7, 8, 9, 10, 16, 17 and 18; on the day 24 the dose intake was higher than day 2, 4, 7, 8, 10, 16 and 18 and on the day 26 the cocaine intake was higher than the day 4 and 8 ($F_{(26,571)}$ = 1.791, P < 0.001).

The statistical analyses detected that the F *Sham* group had more cocaine intake than F 6-OHDA and M *Sham* on the days 13(P = 0.003 and P = 0.013), 23 (P = 0.002 and P = 0.009), 24 (P = 0.029 and P = 0.017) and 27 (P = 0.029 and P = 0.022) ($F_{(78,571)}$ = 1.791, P = 0.035).

Subunits $GABA_A$ mRNA expression

There was no difference in PFC α_1, α_2 and γ_2 subunit mRNA expression between groups. Nevertheless, we found a strong positive correlation between α_1/α_2 (r = 0.860; *P*= 0.013) and $\alpha 1/\gamma 2$ (r = 0.834; P 0.020) within F *Sham* group. Furthermore, the analyzed showed that there is a strong positive correlation between α_2 mRNA expression with reinforcement (r = 0.828; *P* = 0.042).

Discussion

According to studies in humans with ADHD, who present higher risk of cocaine self-administration, we expected to see higher cocaine reinforcement in our model with 6-OHDA-lesioned animals [27,28,29]. Our data are different from our expectations. In this study we showed that male and female rats in the ADHD model do not present higher reinforcement in an oral cocaine operant self-administration paradigm in comparison to non-lesioned animals.

It was observed that normal male rats increased lever pressing in the oral cocaine self-administration paradigm and when higher doses were presented the lever presses decreased. Other study in normal rats indicates that the number of lever presses decreases with the increase of cocaine concentration in e.v. self-administration [58]. Thus, the animals compensate the increase of the dose with the decrease of lever presses in order to maintain a specific concentration of drug in blood and brain [59]. In ADHD males the reinforcing pattern is lower than in normal males and does not change with the increase of cocaine concentration, ie, 6-OHDA males do not significantly alter the demand for reinforcement. Insufficient dopamine in the mesolimbic region decreases cocaine reinforcement [60, 61]. Bilateral 6-OHDA-induced lesions into the nucleus acumbens produced large and long-lasting decreases in operant responding for cocaine [60].

Differently from the male rats, both non-lesioned and 6-OHDA-lesioned females present similar cocaine reinforcement. Therefore, in females the dopamine insufficiency does not alter responses to different doses cocaine in the oral self-administration paradigm. In line with epidemiological data on human and preclinical findings from animals, the ADHD female rats appear to be more sensitive to the reinforcing effects of cocaine as reflected by the acquisition of self-administration [10; 63]. In our study, ADHD female rats, present higher reinforcement in oral cocaine self-administration than the ADHD males. With these data we can speculate that the brain of female rats can be protected of neurotoxin or there is a difference in neuromodulation of the reward system with the growth of females and consequent release of female sex hormones [40].

As reviewed in other studies [62] one would expect that normal non-lesioned females would present higher lever-press responding than the males. This was not

the case with the oral cocaine self-administration. Male rats responded at higher rates than females. Other studies have also failed to observe the female supremacy in cocaine consumption and different explanations are presented referring to methodological issues associated with the observation of the differences between sexes, such as cocaine doses, the fixed ratio (FR1) of reinforcement and the complexity of testing schedules [63, 64]. On the other hand, the daily amount of cocaine consumed increased faster and was higher for non-ADHD females than their male counterparts, confirming the results that implicate the female gonadal hormones on the biological basis for higher behavioral sensitization, faster acquisition and higher conditioned rewarding effects of cocaine in females [10, 12, 17 , 31, 62].

Our results show a pattern of simple inverse relationship between lever pressing behavior and the total amount of cocaine injected, ie, a dose-dependent decrease in lever pressing behavior occurred as the dose of cocaine was increased [64]. The present study shows that when the concentration of cocaine was increased to 0.3 mg / mL all groups, with exception of 6-OHDA-lesioned females, they increase the cocaine intake. The normal male rats and 6-OHDA-lesioned males seem to differ in daily dose of cocaine only when the cocaine concentration is lower, the intermediate concentration of these animals have the same pattern daily dose of cocaine consumed. This is not seen between normal and 6-OHDA-lesioned females, which begin to differentiate at this moment, with normal non lesioned females having higher consumption than the ADHD females.

Some studies of cocaine self administration were conducted to date in animals induced to ADHD by intracisternal injection of 6-OHDA. One of the studies that corroborate our results, it was used the bilateral 6-OHDA injection directly model into the ventral tegmental area, and showed that the lesioned male decrease cocaine infusions when compared to non-lesioned males [42]. In one study that the rats received either bilateral injections of 6-OHDA into the medial prefrontal cortex (mPFC) after stable response patterns were obtained, the lesions did not affect either the rate or pattern of intravenous cocaine self-administration,ie, the lesion fail to influence intravenous self-administration of cocaine, suggesting that mPFC does not appear to be a critical substrate for the maintenance of cocaine self-administration [55]. Other study measured the effect of 6-OHDA lesion of the *nucleus accumbens* (NAc) on lever-press behavior before and during cocaine self-administration. Its results showed that destruction of DA terminals in the NAc decreased the total intake

of cocaine during the self-administration sessions and impaired discriminative lever-responding for the drug, both during cocaine self-administration. It is concluded that DAergic systems in the NAc might be involved in the reinforcement and/or motivational processes underlying cocaine self-administration [56].

The ADHD model of neonatal 6-OHDA lesion is more often studied in male rodents and is known to induce hyperactive in comparison to sham-lesioned rats. Several studies showed that the presynaptic neurotoxin 6-OHDA administered intraventricularly to neonatal rats causes considerable and long-lasting locomotor hyperactivity in juvenile and adult rats [24, 36, 25, 7,2]. In our case the increased locomotion seems to be mainly seen in males, while females are naturally more active rendering that 6-OHDA-lesioned and normal non-lesioned rats present similar high locomotor activity, as described by other authors [26,37]. The lack of behavioral motor activity change in females due to 6-OHDA may be due to sex-specific effects of 6-OHDA and female sex hormones. In humans, most girls/women with ADHD do not show motor hyperactivity as intense as seen in boys/men [26].

Unlike the results found previously in our lab, where cocaine had a paradoxical effect on the 6-OHDA-lesioned male rats, reversing the motor hyperactivity when cocaine was consumed orally in a two-bottle choice paradigm, in this study we found an increase in locomotor activity after oral cocaine [2]. Probably, this occurred because of the methods differences. In the previous study the two bottle choice technique was used for oral cocaine self-administration and open field test for examining the locomotor activity of the animals, while in our study we used the operant chambers for cocaine self-administration and the horizontal chambers to measure locomotor activity. The two bottle choice method allowed the animals to consume around 3 times less cocaine [2] than in the present study with the operant behavior. Because the animals were more reinforced and received higher doses of cocaine over a shorter period of time with the operant self-administration in this study, one may propose that the paradoxical effect was not observable due to the higher doses of cocaine consumed. Another study that performed the same dopaminergic lesion showed that low doses of methylphenidate reduces hyperactivity of lesioned rats by neurotoxin 6-OHDA. The other hand, these authors found that higher doses of methylphenidate produced potentiated levels of locomotion [38]. Other studies have tested the impaired locomotor activity after psychostimulant administration. A study carried out injection directly into 6-OHDA mCPF and found that the locomotor

response was dose-dependent in these animals with lesions, ie, since the dose of cocaine is increased, the locomotor activity increases [51]. In another study, the injection was made directly in the caudate nucleus, and after amphetamine injection locomotor activity decreased, however after 14 days of psychostimulant administration locomotor activity increased to levels similar to those of the sham group. [52]

The paradoxical effects may be due to increased extracellular dopamine concentration caused by psychostimulant which can reverse the hypofunction of the mesolimbic dopaminergic pathway destroyed by 6-OHDA, thus attenuating hyperactivity model of ADHD [22]. Moreover, the results of our study showing the increased mobility after a long period of high doses of cocaine self-administration might be explained by the fact that 6-OHDA have only 20% of intact dopaminergic neurons, rendering higher susceptibity to dopamine binding [53,57]. Therefore higher doses of cocaine inhibit reuptake and allow higher extrasynaptic concentration of dopamine and, consequently, greater chances for dopamine binding to upregulated D-2 [53,54] receptors, causing increased locomotor response to the psychostimulant.

Although there are no significant differences between ADHD and normal animals of the different GABA subunits, we can see that, except for $\alpha 2$ subunits in male rats, the mRNA subunit expressions are lower when the animals are lesioned. Even though we cannot see a significant change in mRNA expression, it may be suggested that the increase in the number of animals per group would show decreased expression of $\alpha 1$ and $\gamma 2$ subunits in the prefrontal cortex in ADHD males, as well as an increase expression of $\alpha 2$ mRNA. This apparent increase may be caused by high doses of cocaine consumed for animals, unlike the previous study from our laboratory where there was an attenuated of expression in self-administration of low doses of cocaine in ADHD males [2]. Our study showed differences between the groups when considering the correlations between the subunits and cocaine reinforcement. Only normal non-lesioned females demonstrated the correlation subunit $\alpha 1$, $\alpha 2$ and $\gamma 2$. Thus, it can be suggested that the lesion with 6-OHDA in females interfere in correlation expressions of $GABA_A$ subunits. Do not has correlation in lesioned males, thus it is suggested that the finding between sham females after use of high doses of cocaine is related to the presence of female sex hormones. With our results, we cannot consider that cocaine

increases the expression of $GABA_A$ subunits in the non-lesioned animals after high doses of cocaine, but for most sex pairs lesioned or non-lesioned, these animals seems to increase expression, contrary to what was seen in a study where the GABA receptors of intact rats decreased of expression subunits, after repeated injections of cocaine [43,44]. Subunits $GABA_A$ expression may change after exposure to cocaine, due the intense release of dopamine. Several studies show that there may be increased or decreased subunits $GABA_A$ expression depending of the brain region analyzed and the time of exposure to cocaine. For example, 1 h after the injection of cocaine in cortex, cerebellum and striatum there is an increase of $\alpha 1, \beta 2$ and $\beta 3$-$GABA_A$ mRNA [45]. In other hand, after 14 days chronic treatment with cocaine, the subunit $\beta 3$ $GABA_A$ expression was decreased in the cortex, caudate and putamen [46]. 6-OHDA lesioned male rats exposed to 23 days chronic treatment with cocaine had decrease subunit $\alpha 2$, $\alpha 4$ and $\beta 2$ $GABA_A$ expression in the prefrontal cortex, while $\beta 2$ subunit was attenuated after oral cocaine. We cannot assert that the sham animals in this study had changes in mRNA expression after cocaine self-administration because we do not have non-lesioned group that not receiving cocaine, but studies have shown that the subunits $\alpha 1$ and $\alpha 2$ are the most involved in behavior after the use of drugs in non-lesioned animals [47,48]. Between ADHD and normal males there was also difference in the correlation between variables. Contrary to what happened in females, ADHD animals showed correlation between variables. In these animals, the expression of $\alpha 2$ subunit is positively correlated with reinforcement. This is in accordance with the literature because studies show that the $\alpha 2$ subunit has very important role in drug addiction, particularly cocaine, because it is directly involved in reward-related behaviors such as behavioral sensitization to psychostimulants [49,50].

Therefore, in this other study in our laboratory was seen that hyperactivity of 6-OHDA-lesioned rats is associated with alterations in the functioning of the GABAergic system suggesting that cocaine at low doses reverses behavioral changes in animals hyperactive attenuate defects in the inhibition system induced by 6-OHDA [2], so in our case, it is suggested that high doses of cocaine self-administered in 6-OHDA-lesioned animals do not have the capacity to attenuate the GABA system defects generated by 6-OHDA.

Conclusion

Our results provide important information about differences in drug reinforcing patterns in animals, males and females, induced to ADHD and the implication of sex on these patterns. We also show that the paradoxical effect of cocaine does not happen when there is a high consumption of this substance by ADHD animals. While we cannot add clear information about the higher doses of cocaine effects on the $GABA_A$ receptor subunits expression, our findings provide clues that there may be changes caused by cocaine use in animals that have dopaminergic lesions. Therefore, there is need for more studies to verify self-administration of cocaine through different routes in male and female animals induced to ADHD and how changes in $GABA_A$ subunit expression may be related to psychostimulants addiction.

Conflict of interest

The authors have no conflict of interest.

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Legends

Figura1: Differences in locomotor activity responses of young adult 6-OHDA/*Sham* during 15 min test in female and male rats. Locomotor activity was significantly increased in 6-OHDA lesioned rats. Means ± SE are presented. * $P = 0.021$.

Figure 2: Locomotor activity after oral cocaine self-administration. Locomotor activity was significantly different in the 26th day from baseline in M 6-OHDA, M *Sham* and F *Sham* * $P < 0.05$. Means ± SE are presented.

Figure 3: Daily of number of infusions mean was lower in 6-OHDA lesioned rats. * $P = 0.013$. Means ± SE are presented.

Figure 4: Daily lever pressing for cocaine solution delivery mean per concentration. * $P < 0.001$, different from M *Sham.* ** $P < 0.001$, different from M 6-OHDA. ^^ $P =$

0.003, different from F *Sham*. Means ± SE are presented.

Figure 5: Daily cocaine intake mean per group during 3 hours. * $P < 0.05$, different from F 6-OHDA and M *Sham*. ^ $P < 0.05$, different from day 1, 2, 3, 4, 6, 7, 8, 9, 10, 16, 17 and 18. $^{o}P < 0.05$, different from day 2, 4, 7, 8, 10, 16 and 18. ^^ $P < 0.05$, different from day 4 and 8. $^{\#}P < 0.05$, different from day 2, 4, 7, 8, 10, 16 and 18. Means ± SE are presented.

Figure 6: The subunits $GABA_A$ mRNA expression analyses in the prefrontal cortex in the different groups after cocaine self-administration. Means ± SE are presented.

Table 1: Pearson correlation between the analyzed parameters of Female Sham and Female 6-OHDA groups: Basal locomotion, Locomotion 26th, Reinforcement, γ2, α1 and α2 mRNA expression.

Table 2: Pearson correlation between the analyzed parameters of Male Sham and Male 6-OHDA groups: Basal locomotion, Locomotion 26th, Reinforcement, γ2, α1 and α2 mRNA expression.

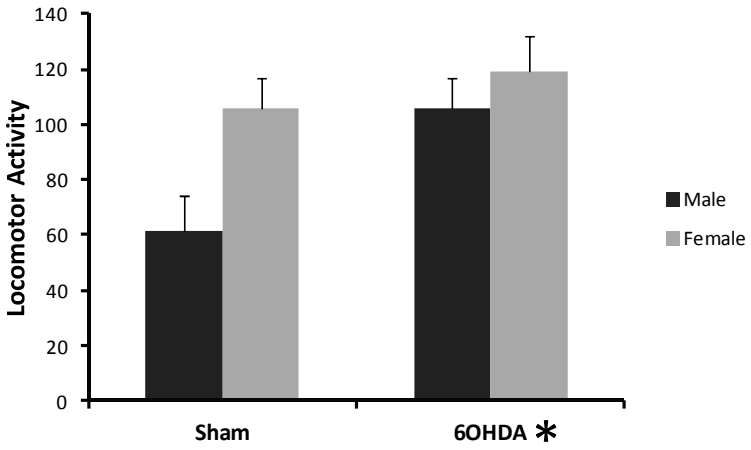

Fig1: Differences in locomotor activity responses of young adult 6-OHDA/*Sham* during 15 min test in female and male rats. Locomotor activity was significantly increased in 6-OHDA lesioned rats. Means ± SE are presented. * $P = 0.021$.

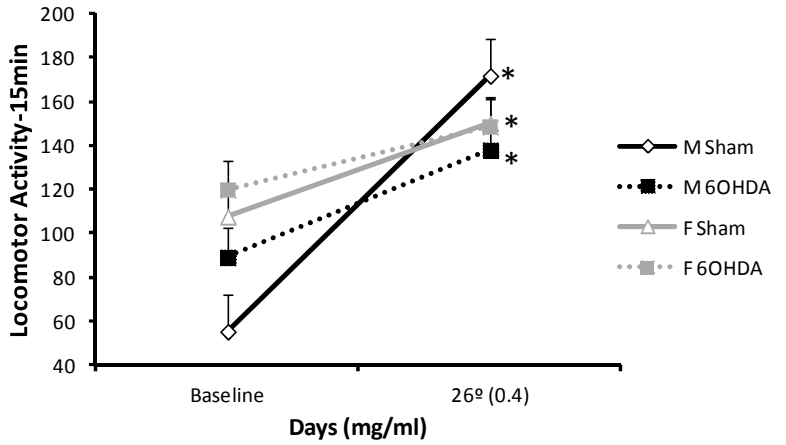

Fig 2: Locomotor activity after oral cocaine self-administration. Locomotor activity was significantly different in the 26th day from baseline in M 6-OHDA, M *Sham* and F *Sham* * $P <$ 0.05. Means ± SE are presented.

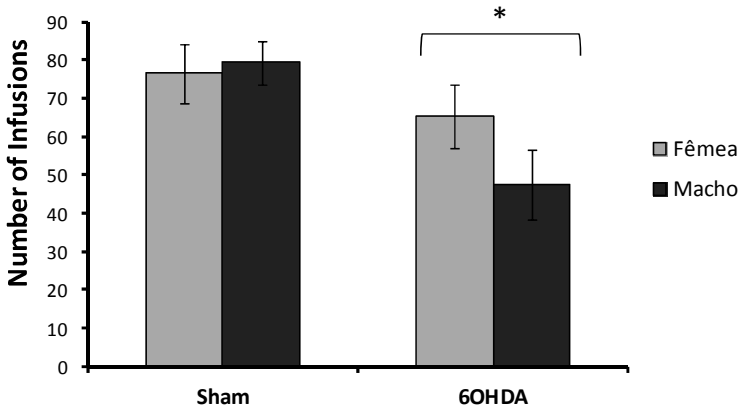

Fig 3: Daily of number of infusions mean was lower in 6-OHDA lesioned rats. * *P* = 0.013. Means ± SE are presented.

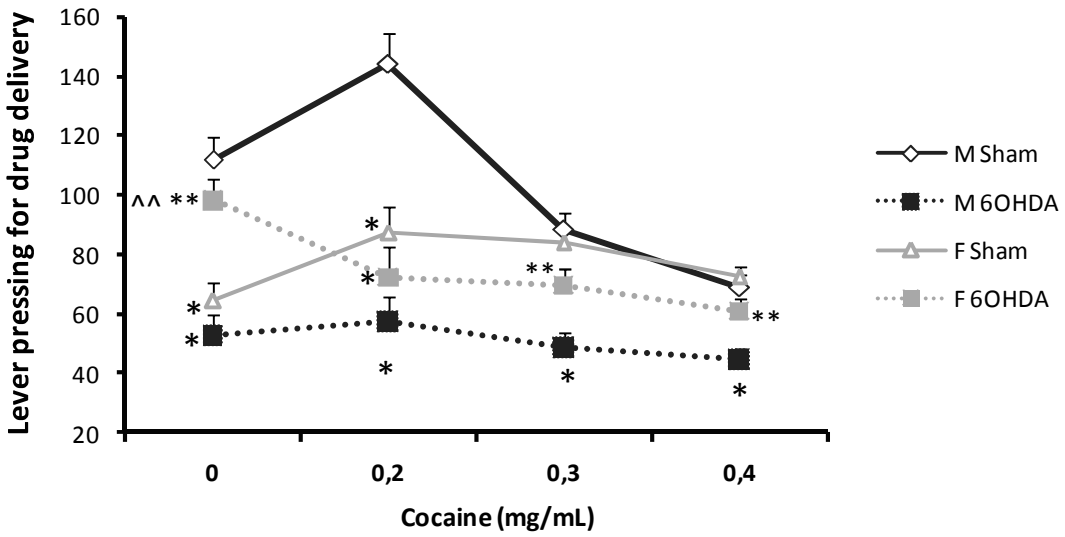

Fig 4: Daily lever pressing for cocaine solution delivery mean per concentration. * *P* < 0.001, different from M *Sham.* ** *P* < 0.001, different from M 6-OHDA. ^^ *P* = 0.003, different from F *Sham*. Means ± SE are presented.

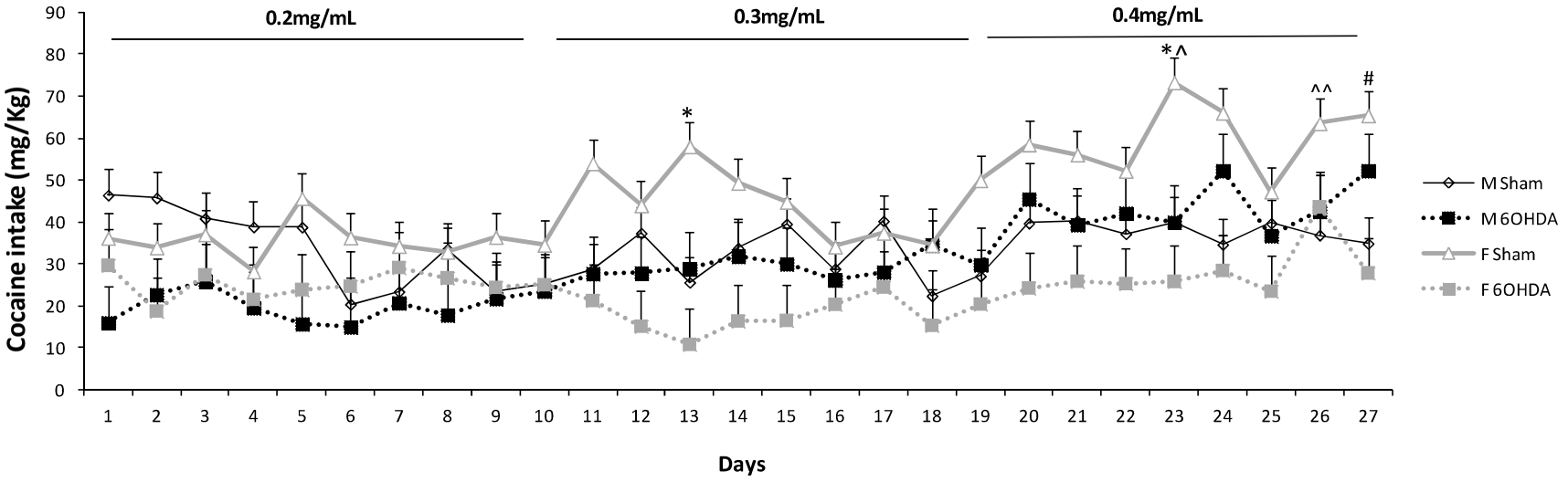

Fig 5: Daily cocaine intake mean per group during 3 hours. * *P* < 0.05, different from F 6-OHDA and M *Sham*. ^ *P* < 0.05, different from day 1, 2, 3, 4, 6, 7, 8, 9, 10, 16, 17 and 18. °*P* < 0.05, different from day 2, 4, 7, 8, 10, 16 and 18. ^^ *P* < 0.05, different from day 4 and 8. # *P* < 0.05, different from day 2, 4, 7, 8, 10, 16 and 18. Means ± SE are presented.

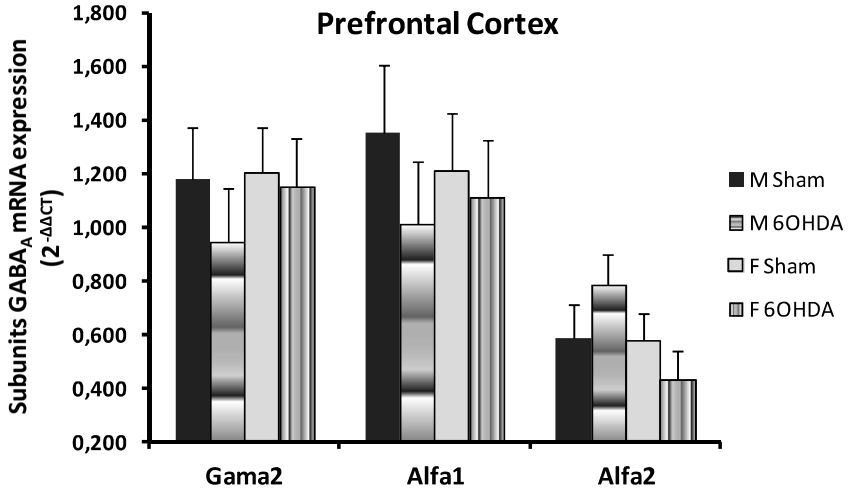

Fig 6: The subunits $GABA_A$ mRNA expression analyses in the prefrontal cortex in the different groups after cocaine self-administration. Means ± SE are presented.

Table 1: Pearson correlation between the analyzed parameters of Female Sham and Female 6-OHDA groups: Basal locomotion, Locomotion 26th, Reinforcement, γ2, α1 and α2 mRNA expression.

	F *Sham*					F 6-OHDA				
	Locom. 26th	**Reinforc.**	**γ2**	**α1**	**α2**	**Locom. 26th**	**Reinforc.**	**γ2**	**α1**	**α2**
Basal Locom.										
r	-0,008	-0,015	-0,558	-0,666	-0,043	0.679	0.357	-0.226	-0.093	0.250
P	0,982	0,967	0,250	0,149	0,936	0.064	0.385	0.667	0.842	0.589
Locom. 26th										
r		0,393	-0,151	-0,395	-0,530		0.245	-0.385	-0.607	-0.168
P		0,231	0,746	0,380	0,221		0.559	0.450	0.148	0.720
Reinforc.										
r			0,136	0,166	0,623			0.125	-0.316	-0.091
P			0,771	0,721	0,135			0.813	0.490	0.846
γ2										
r				0,860	0,726				0.175	-0.808
P				**0,013***	0,065				0.740	0.052
α1										
r					0,834					0.221
P					**0,020***					0.634

Table 2: Pearson correlation between the analyzed parameters of Male Sham and Male 6-OHDA groups: Basal locomotion, Locomotion 26th, Reinforcement, γ2, a1 and a2 mRNA expression.

		M *Sham*					M 6OHDA				
		Locom. 26º	**Reinforc.**	**γ2**	**α1**	**α2**	**Locom. 26º**	**Reinforc.**	**γ2**	**α1**	**α2**
Basal Locom.											
	r	-0,720	-0,252	-0,351	0,438	0,579	-0,350	-0,423	0,824	0,280	-0,414
	P	0,170	0,547	0,563	0,461	0,306	0,395	0,224	0,086	0,591	0,415
Locom. 26º											
	r		-0,047	-0,429	-0,820	0,435		-0,032	-0,077	-0,231	-0,267
	P		0,941	0,718	0,388	0,713		0,940	0,902	0,660	0,609
Reinforc.											
	r			0,322	0,090	-0,213			-0,575	-0,059	0,828
	P			0,597	0,885	0,731			0,311	0,912	**0,042***
γ2											
	r				0,642	-0,136				0,674	-0,431
	P				0,243	0,827				0,213	0,469
α1											
	r					0,554					-0,133
	P					0,333					0,802

6 CONSIDERAÇÕES FINAIS

O presente estudo fornece informações importantes quanto aos padrões de reforço de cocaína em animais induzidos ao transtorno e a implicação do sexo nesses padrões. Além disso, verificou-se que a autoadministração oral de cocaína afetou de maneira diferente os comportamentos de locomoção dos animais induzidos ao TDAH por 6-OHDA. Já alterações neuroquímicas entre os grupos estudados não foram encontradas. Embora não possamos acrescentar informações claras sobre os efeitos da cocaína na expressão das subunidades do receptor $GABA_A$, nossos resultados fornecem pistas de que pode haver modificações importantes ocasionados pelo consumo de cocaína em animais machos que têm lesões dopaminérgicas. Com este estudo verificamos que o efeito paradoxal da cocaína não acontece quando há um consumo de altas doses dessa substância pelos animais lesionados. Portanto percebe-se a necessidade de mais estudos que verifiquem o consumo de cocaína em animais machos e fêmeas induzidos ao TDAH e as alterações da expressão de subunidades do $GABA_A$ relacionados com adição a psicoestimulantes para elucidar as bases comportamentais e neuroquímicas da coexistência desses dois transtornos.

7 ANEXOS

7.1 Anexo I

7.1.2 COCAÍNA – REVISÃO DA LITERATURA

Epidemiologia

Segundo o relatório mundial sobre drogas da Organização das Nações Unidas (ONU), publicado em 2014, o uso mundial de cocaína se manteve estável em 2012, com uma estimativa de 14 milhões a 21 milhões de usuários (prevalência anual de 0,4%). O uso de cocaína se manteve elevado na América do Sul (1,8%), Oceania (1,5%) e Europa Ocidental e Central (1%). Já na América do Norte não houve aumento no uso de cocaína entre 2011 e 2012. Em relação ao número de usuários de cocaína na América do Sul, houve um aumento quando se compara os anos de 2004 e 2005 com o ano de 2012 (2 milhões para 3,3 milhões). A influência desse aumento pode ser devido às várias formas de uso, incluindo o *crack* e outras formas brutas de base de cocaína.

De acordo com o II Levantamento Domiciliar sobre o Uso de Drogas Psicotrópicas no Brasil, que envolveu as 108 maiores cidades do país realizado em 2005, 2,9% da população já fez uso de cocaína pelo menos uma vez na vida, o que equivale a 1.459.000 pessoas. Dentre os entrevistados, 0,5% eram da faixa etária de 12 a 17 anos, 4,2% de 18 a 24 anos, e a maior parte, 5,2%, de 25 a 34 anos. Nas duas maiores faixas etárias, o sexo masculino obteve grande predomínio, diferentemente da faixa etária entre 12 a 17 anos em que a porcentagem entre os dois sexos se igualou (0,4%). Na região sul a porcentagem dos entrevistados que já fizeram uso de cocaína na vida chegou a 3,1% e, assim como observado no país inteiro, o consumo entre o sexo masculino prevalece (CARLINI *et al., 2007*).

Entre os estudantes brasileiros do ensino fundamental e médio, segundo estudo realizado em 2010 (CARLINI *et al, 2010*), 2,5% deles já fizeram uso na vida

de cocaína, observando-se um aumento de 0,5% quando comparado ao estudo feito anteriormente, em que se contabilizou 2% dos estudantes (GALDURÓZ *et al., 2005*).

Um estudo feito pela FIOCRUZ em 2012 nas capitais do Brasil e no Distrito Federal revelou que existe uma proporção de 0,81% da população que consomem *crack* ou similares, representando cerca de 370 mil usuários. Dentre eles, cerca de 0,11% eram crianças e adolescentes, ou seja, em torno de 50 mil crianças e adolescentes fazem uso dessa droga nas capitais do país.

Farmacologia

A cocaína é purificada a partir das folhas de coca, as quais contêm de 0,6 a 1,8% do alcalóide, através de um método relativamente simples, pelo qual esta é extraída das folhas utilizando um solvente orgânico (querosene ou éter).

Existem quatro formas diferentes de apresentação da cocaína: basuco (ou pasta base), a forma de pasta de coca; merla, a forma pastosa; cloridrato de cocaína, a forma de pó cristalino; crack, a forma de base livre, sendo as duas últimas formas de uso mais populares no sul do país.

A forma de cloridrato de cocaína é obtida através do tratamento da pasta de coca purificada com ácido clorídrico. A dissolução do alcalóide no ácido forma um pó hidrossolúvel, que precisa ser recristalizado, através de aquecimento, para que o produto final seja o pó de cloridrato de cocaína, o qual pode ser ingerido via oral, aspiração nasal ou via intravenosa. Esta forma não pode ser fumada, pois, com o aumento da temperatura, ocorre a volatilização e conseqüente decomposição do composto (CHASIN et al., 2008; DICKERSON & JANDA, 2005).

O *crack*, base livre, é preparado através do aquecimento da solução aquosa do cloridrato com uma substância básica, como o bicarbonato ou o hidróxido de

sódio, neutralizando o bicarbonato ou a parte ácida. Com o aquecimento e posterior resfriamento, precipita-se a base livre, com aspecto de cristais irregulares em forma de pedras. Esta forma é estável a altas temperaturas, apresentando um baixo ponto de fusão, o que lhe permite ser fumado, após pirólise, inalando-se os vapores (CHASIN et al., 2008; DICKERSON & JANDA, 2005).

Farmacocinética

A absorção da cocaína varia de acordo com a via de introdução, que pode ser intranasal (aspiração), intravenosa, respiratória (inalação pelo ato de fumar) e oral (CHASIN et al., 2008). Quando a cocaína é aspirada, a absorção ocorre pela mucosa nasal, e sua velocidade é relativamente baixa, devido às propriedades vasoconstritoras da droga, e lenta, permitindo teores plasmáticos menores por tempo mais prolongado (CHASIN et al., 2008). Cerca de 20% a 30% da droga são absorvidos pela mucosa nasal (FIGLIE *et al*., 2004). Se injetada (via intravenosa), a droga alcança a corrente sanguínea imediatamente, e sua biodisponibilidade é de 100%. Os efeitos são muito intensos e rápidos. A via respiratória (fumada) pode ser comparada a esta via em termos de velocidade de absorção, pico de concentração plasmática, duração e intensidade dos efeitos (CHASIN et al., 2008; FIGLIE *et al*., 2004). Quando a cocaína é ingerida por via oral, os feitos aparecem em um maior tempo, pois sua absorção é lenta e incompleta (FIGLIE *et al*., 2004). Isso é explicado pela ionização da cocaína no meio ácido do estômago e a demora em atingir o meio menos ácido do intestino delgado, onde a forma não-ionizada prevalece, levando a uma maior velocidade de absorção (CHASIN et al., 2008). Por esta via somente 25% da droga ingerida alcançam o cérebro. Esses motivos explicam a ausência do efeito *rush*, caracterizado por uma intensa sensação de bem estar, comum às outras

formas de uso citadas. No entanto, é a via em que os efeitos persistem por mais tempo. O início, o pico e a duração dos efeitos da cocaína de acordo com a via podem ser melhor entendidos na tabela 1.

Tabela 3. Farmacocinética da cocaína de acordo com a via de administração.

Via de administração	Início da ação	Pico de efeito	Duração da ação
Inalada/fumada	3-5 segundos	1-3 minutos	5-15 minutos
Intravenosa	10-60 segundos	3-5 minutos	20-60 minutos
Intranasal ou mucosal	1-5 minutos	15-20 minutos	60-90 minutos
Gastrointestinal (oral)	> 20 minutos	> 90 minutos	> 180 minutos

Fonte: EGRED & DAVIS, 2005.

Após penetrar no cérebro, a cocaína é redistribuída para outros tecidos, se concentrando no baço, rins, fígado e cérebro. O acúmulo no cérebro se deve ao caráter lipofílico da cocaína, permitindo que a substância atravesse a barreira hematoencefálica, seja seqüestrada pelos adipócitos, e então se acumule no sistema nervoso central. Essa característica também explica a transferência da droga pela placenta através de transporte passivo, assim como na secreção láctea (FIGLIE *et al*., 2004; CHASIN et al., 2008).

A eliminação da cocaína é em sua maior parte controlada pela sua biotransformação, a qual é muito extensa devido às características da molécula, resultando em menos de 10% da cocaína excretada sem alteração na urina. Assim, cerca de 90% é transformado em metabólito inativo hidrossolúvel. Após absorvida a cocaína (benzoilmetilecgonina) inicia o metabolismo no plasma por hidrólise do

radical éster, produzindo éster metilecgonina. Outro metabólito produzido é a benzoilecgonina que é originada pela hidrólise espontânea ou por reação catalisada pelas carboxilesterases. Por último, ocorre a desmetilação da cocaína realizada no fígado pela enzima oxidase, resultando na norcocaína, um produto farmacologicamente ativo (FIGLIE *et al*., 2004; CHASIN et al., 2008).

A eliminação da cocaína é feita rapidamente através da urina. O tempo de meia-vida é próximo de 1 hora (FIGLIE *et al*., 2004). Em torno de 1 a 5% da substância não é alterada e os metabólitos hidrossolúveis da cocaína fornecem um indicador do uso recente, pois podem permanecer na urina por até 72 horas (EGRED & DAVIS, 2005). Quando se trata de indivíduos em uso crônico a urina pode permanecer positiva por até 22 dias (FIGLIE *et al*., 2004).

Farmacodinâmica

A cocaína é um potente agente simpaticomimético, por sua capacidade de bloquear a recaptação nos neurônios pré-sinápticos, aumentando os níveis de catecolaminas (dopamina, noradrenalina e serotonina), produzindo elevação temporária da concentração desses neurotransmissores nos receptores pós-sinápticos, justificando os efeitos estimulantes do Sistema Nervoso Central (SNC) (EGRED & DAVIS, 2005; CHASIN et al., 2008).

O principal mecanismo de ação no SNC é o bloqueio da recaptação da dopamina (DA) nas fendas sinápticas, que ocorre devido à ligação da cocaína à bomba de recaptação de dopamina, inibindo a remoção deste neurotransmissor da fenda sináptica. Com isso, a DA permanece na fenda, ligando-se livremente aos seus receptores da membrana pós-sináptica, produzindo mais impulsos nervosos. A concentração de DA no núcleo *accumbens* (NAc), com esse bloqueio, pode

aumentar em até 15 vezes (GRIMM, 2007), e esse aumento parece estar envolvido de forma importante na recompensa e recaída (WISE, 1996). A recaptação de DA ocorre principalmente na via mesocorticolímbica, uma das principais responsáveis pelo efeito reforçador da cocaína e pelo sentimento de extrema euforia (GOLDSTEINS & VOLKOW, 2002; KOOB, 2006; KOOB & MOAL, 2006), a qual se origina na área tegmental ventral e projeta-se para áreas do sistema límbico, incluindo o NAc , o córtex pré-frontal, o hipocampo e a amígdala (ANDERSON & PIERCE, 2005; KOOB & MOAL, 2006). Outra via importante é a nigro-estriatal onde encontra-se cerca de 75% da DA no cérebro e cujos corpos celulares se situam na substância negra e axônios terminam no corpo estriado. O sistema túbero-hipofisário é uma terceira via de relevância, no qual seus neurônios seguem trajeto do hipotálamo para a eminência mediana e a hipófise (CHASIN et al., 2008). Sendo assim, a DA tem um papel crucial na mediação dos efeitos reforçadores da cocaína (AARÃO et al., 2008; CANNON & BSEIKRI, 2004).

O aumento da concentração de DA causado pelo bloqueio da recaptação deste neurotransmissor gera no sistema neural adaptações para neutralizar o efeito da cocaína (WISE & KOOB, 2014). Essas neuroadaptações ocorrem tanto no próprio sistema dopaminérgico como nos outros sistemas neurais (KOOB AND BLOOM, 1988) podendo acarretar profundas alterações em receptores, síntese de neurotransmissores como também em níveis moleculares e genéticos (JOHANSON & SCHUSTER, 2000; PERROTTI e cols., 2000). O processo de neutralização do efeito da cocaína, ou seja, a tentativa de equilibrar a intensa estimulação do SNC, ocorre com a mobilização dos sistemas inibitórios cerebrais, principalmente o sistema GABAérgico. Como consequência da administração crônica de cocaína, a

expressão das subunidades dos receptores $GABA_A$ pode ser alterada (Suzuki et al., 2000; Yamaguchi et al., 2000, 2002)

Efeitos da cocaína

A cocaína possui múltiplos efeitos, devido às ações tanto periféricas, quanto centrais (LARANJEIRA *et al*., 2003). Os efeitos da intoxicação aguda são intensa euforia e bem estar, aumento do estado de vigília, autoconfiança elevada e aceleração do pensamento. Como principais sintomas físicos podem ser citados o aumento da frequência cardíaca e respiratória, da temperatura corporal, sudorese, tremor leve de extremidades, espasmos musculares, midríase e comportamento estereotipado repetitivo. Podem ocorrem também quadros de pânico, transtornos depressivos e psicose, os quais são as complicações psiquiátricas mais relatadas. Já entre as não-psiquiátricas, as complicações cardiovasculares são as mais freqüentes (LARANJEIRA *et al*., 2003). Tais eventos são diretamente proporcionais a dose da droga ingerida e podem variar, de acordo com variações orgânicas individuais.

A cocaína é considerada uma droga com grande potencial de abuso e dependência devido a sua grande capacidade de produzir o efeito desejado, chamado de reforço positivo, cuja atribuição se dá à potenciação da neurotransmissão dopaminérgica dos neurônios da região mesocorticolímbica (CHASIN et al., 2008). Para que um indivíduo receba um diagnóstico de dependência, segundo os critérios do Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-V) (APA, 2014) é preciso preencher alguns dos seguintes sintomas: tolerância, abstinência, consumo de substâncias em maiores quantidades ou por um período mais longo do que o pretendido, desejo persistente ou esforços

mal-sucedidos em reduzir ou controlar o uso, longo tempo gasto para obtenção da droga, na sua utilização ou na recuperação dos efeitos, abandono ou redução das atividades sociais, ocupacionais ou recreativas para uso da substância, uso da substância contínuo ou recorrentemente, mesmo com a consciência do problema, fissura, uso recorrente em situações que envolvem risco à integridade física.

Diferença entre os sexos no uso de cocaína

Dados coletados entre 2004 e 2009 do sistema de saúde pública que monitora os atendimentos relacionados a drogas nos Estados Unidos relataram que nos atendimentos a mulheres, a cocaína é a droga ilícita mais frequentemente envolvida na procura pela emergência (53,7%) (SAMHSA, 2011). Os últimos estudos epidemiológicos feitos no Brasil, em 2007, mostraram que na faixa etária entre 18 e 34 anos, o consumo de cocaína ainda é maior entre os homens. No entanto, quando se avalia a faixa da adolescência, entre 12 e 17 anos, o consumo entre os dois sexos se iguala (CARLINI *et al., 2007*).

As mulheres geralmente iniciam o uso de cocaína mais precocemente e com uma maior freqüência (LYNCH *et al*., 2002), tendem a progredir do primeiro uso à dependência mais rapidamente (SAMHSA, 2011) e enfrentam normalmente um padrão mais grave de dependência (CARROL *et al., 2004;* HU E BECKER, 2008; KERSTETTER AND KIPPIN, 2011). Outro aspecto importante é a maior tendência das mulheres dependentes em adquirir doenças de saúde mental e maior propensão em tentar o suicídio em relação aos homens (KERSTETTER AND KIPPIN, 2011; SAMHSA, 2011). Muitos estudos, tanto em animais quanto em humanos, demonstram significativa interferência dos hormônios sexuais na ação em sistemas neuroquímicos (PERROTI et al, 2000; SALEH, 2003; SOUZA et al, 2010), bem como

sobre os efeitos comportamentais (KOURI et al., 2002; LYNCH et al., 2002). A diferença sexual na dependência à cocaína pode ser explicada pelos efeitos dos hormônios femininos e suas flutuações ao longo do ciclo reprodutivo. Alguns estudos demonstram que os efeitos positivos da droga dependem da fase do ciclo menstrual, sendo maiores durante a fase folicular, e que o pico do desejo pela droga se dá quando há concentrações elevadas de estrógeno e progesterona (SOFUOGLU et al., EVANS et al., 2002).

7.1.3 Aprovação CEUA

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UFCSPA

A Comissão de Ética no uso de Animais, analisou o Projeto:

Projeto: 13-122 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
HELENA MARIA TANNHAUSER BARROS
LAÍSA DE SIQUEIRA UMPIERREZ

Título: AUTO-ADMINISTRAÇÃO DE COCAÍNA EM RATOS MACHOS E FÊMEAS COM TRANSTORNO DE DÉFICIT DE ATENÇÃO E HIPERATIVIDADE (TDAH) INDUZIDOS POR 6-HIDROXIDOPAMINA(6-OHDA)

Este projeto foi aprovado em seus aspectos éticos e metodológicos. Todo e qualquer alteração do projeto, assim com eventos adversos graves, deverão ser comunicados a esta CEUA.

Porto Alegre, 20 de agosto de 2013.

Katya V. Rigatto
Coordenadora do CEUA
UFCSPA

7.1.4 Normas da revista

BEHAVIOURAL BRAIN RESEARCH

DESCRIPTION.

Behavioural Brain Research is an international, interdisciplinary journal dedicated to the publication of articles in the field of **behavioural neuroscience**, broadly defined. Contributions from the entire range of disciplines that comprise the **neurosciences**, **behavioural sciences** or **cognitive sciences** are appropriate, as long as the goal is to delineate the neural mechanisms underlying behaviour. Thus, studies may range from neurophysiological, neuroanatomical, neurochemical or neuropharmacological analysis of brain-behaviour relations, including the use of molecular genetic or behavioural genetic approaches, to studies that involve the use of brain imaging techniques, to neuroethological studies. Reports of original research, of major methodological advances, or of novel conceptual approaches are all encouraged. The journal will also consider critical reviews on selected topics.

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